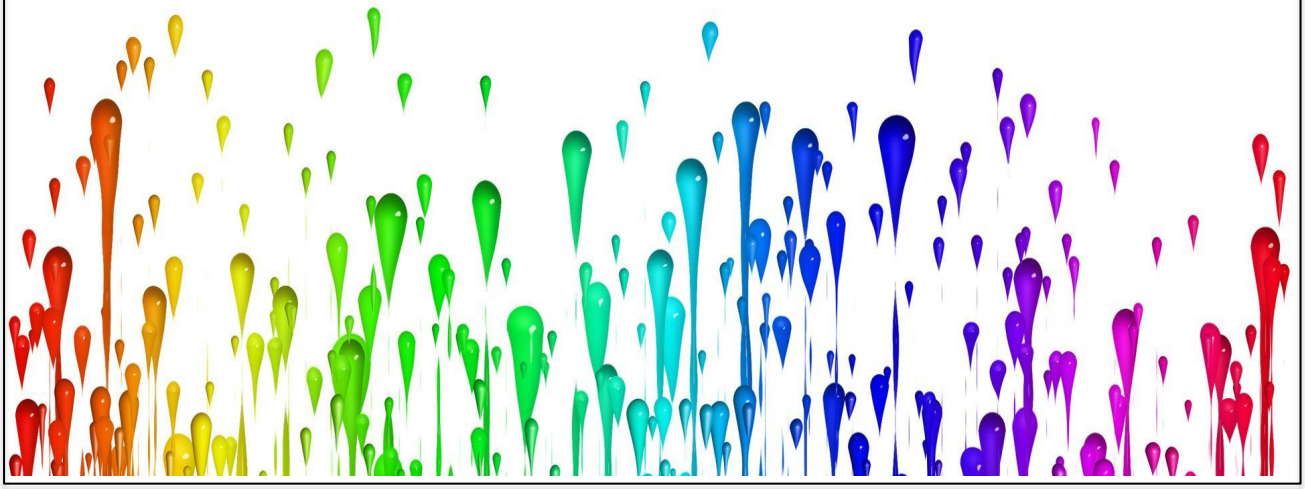
Società Chimica Italiana
Gruppo Interdivisionale
di Biotecnologie(G.I.B.)

Gruppo Interdivisionale di Biotecnologie
Società Chimica Italiana

1° WORKSHOP

I CHIMICI PER LE BIOTECNOLOGIE
Bologna, 23 Febbraio 2018
Dipartimento di Chimica G. Ciamician

Abstracts del convegno



Società Chimica Italiana
Gruppo Interdivisionale di Biotecnologie(G.I.B.)

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO CHIMICA «GIACOMO CIAMICIAN» - CHIM

BtBs Dipartimento di Biotecnologie e Bioscienze Università degli Studi di Milano-Bicocca

UNIVERSITÀ DI PARMA

UNIVERSITÀ DEGLI STUDI DI TRIESTE
Dipartimento di Scienze Chimiche e Farmaceutiche

Università degli Studi di Napoli "Federico II"
Dipartimento di Medicina molecolare e Biotecnologie mediche

PROGRAMMA

Accoglienza Partecipanti: a partire dalle 9.30

INIZIO LAVORI: 10.30

10.30-11.10 Le Biotecnologie e l'industria

10.30-10-50 Dr. Mario Bonaccorso, Assobiotec-Federchimica/Cluster Tecnologico Nazionale della Chimica Verde SPRING "*Bio-based Economy: the role of industrial biotechnology*"

10.50-11.10 Dr. Stefano Donadio, Naicons SRL Milano "*Discovery of bioactive metabolites at NAICONS*"

11.10-12.30-comunicazioni orali

11.10-11.20 Tullia Tedeschi *Playing chemistry with food proteins: molecular tools for food safety, food authenticity and food waste valorization*

11.20-11.30 Elena Cini *Bioconjugation Chemistry for Development of new Antibody Drug Conjugates*

11.30-11.40 Luca D'andrea *Chemical ligation approaches for protein synthesis and modification*

11.40-11.50 Francesco Sansone *Ammonium containing calixarenes as multivalent systems for the delivery of nucleic acids and mimics*

11.50-12.00 Alessandro D'Urso *Porphyrins as versatile tool to design switches, probes and multicomponent arrays with biomolecules*

12.00-12.10 Michael Assfalg *Biomolecular recognition at nanoparticle surfaces: characterization of association equilibria and mapping of binding sites*

12.10-12.20 Barbara La Ferla *Glycotools for biotecnological applications*

12.20-12.30 Roberto Corradini *Design and synthesis of Peptide Nucleic Acids (PNAs) for DNA and RNA-based Therapeutic and Diagnostic Applications.*

12.30-14.00 pranzo

14.00-14.40 LE BIOTECNOLOGIE E L'INDUSTRIA

14.00-14.20 Dr. Paolo Ingallinella - DiaSorin SpA Milano "*Chemistry and biotechnology for immunodiagnostics: a tight synergy for innovation*"

14.20-14.40 Prof. Oreste Piccolo-Studio di Consulenza Scientifica Lecco *"A personal journey in the application of biocatalysis to the synthesis of fine chemicals*

14.40-15.30: COMUNICAZIONI FLASH

14.40 Alessandra Romanelli *Self-assembling nucleic acid analogues for the fabrication of new fluorescent materials*

14.45 Barbara Zambelli *In-cell targeting of activity and maturation of Helicobacter pylori urease as a novel strategy for antibacterial drug screening*

14.50 Antonia Lopreside *Wireless Monitoring of endocrine disrupting chemicals with sensitive bioluminescent yeast biosensor*

14.55 Carlo Morasso *Raman imaging as tool for the label free characterization of cells and tissue*

15.00 Ylenia Beniamino *Nickel effects on human health: NDRG1 and SrnR, two proteins related to Ni2+ dependent tumorigenesis and infections*

15.05 Arrigoni Federica *Quantum Chemistry serving biotechnologies: the interesting case of metalloenzyme active sites.*

15.10 Marco Cespugli *In silico screening of enzymes for polycondensation using structure-based bioinformatic analysis and BioGPS descriptors*

15.15 Simona Fermani *Unravelling the shape and structural assembly of photosynthetic GAPDH complexes from Arabidopsis thaliana*

15.30-16.50 COMUNICAZIONI ORALI

15.30-15.40 Andrea Falanga *PNA-functionalized adenoviral vectors as new tool for gene modulation in anti-cancer treatment*

15.40-15.50 Rebecca Pogni *Ligninolytic enzymes: insights on the catalytic mechanism, enzymatic immobilization and LCA analysis*

15.50-16.00 Valeria Costantino *Biotech applications deriving from Quorum Sensing Studies in marine bacteria*

16.00-16.10 Lucia Gardossi *Chemo-enzymatic valorization of bio-based building blocks for the synthesis of functionalyzed polyesters*

16.10-16.20 Chiara Samorì *Polyhydroxyalkanoates: production, extraction and novel applications*

16.20-16.30 Paola Galletti *Alcohols and amines bio-oxidation using Laccase-mediator system*

16.30-16.40 Maria Calabretta *Improving Cell-Based assays and whole-cell biosensors with bioluminescent 3D cell culture models*

16.40-16.50 Luca Mazzei *Biochemical and structural studies on the inhibition of urease, a nickel-dependent virulence factor*

17.00-17.30 DISCUSSIONE

17.30 Chiusura lavori

MERCK

CESTEV
Centro di Servizio di Ateneo per le Scienze e Tecnologie per la Vita
Università degli Studi di Napoli Federico II

FEDERCHIMICA ASSOBIOTEC
Associazione nazionale per lo sviluppo delle biotecnologie

molecules
an Open Access Journal by MDPI

SPRING
Sustainable Processes and Resources for Innovation and National Growth
Italian Cluster of Green Chemistry

INDICE

Glycotools for biotecnological applications

Quantum Chemistry serving biotechnologies: the interesting case of metalloenzyme active sites.

Arrigoni Federica, Bertini Luca, De Gioia Luca, Zampella Giuseppe

University of Milano-Bicocca, Department of Biotechnology and Biosciences, Piazza della Scienza 2, 20126, Milano, Italy.

e-mail: federica.arrigoni@unimib.it

Chemistry of life involves a huge number of reactions that needs bioinorganic catalysts, such as metalloenzymes.[1] Thanks to these proteins, Nature can accomplish some of the most thermodynamically and kinetically challenging chemical tasks, at outstanding rates, at room pressure and temperature, and by means of only cheap and bioavailable metal ions. Since a longstanding goal in chemistry is the replacement of noble metal-based catalysts with the use of first row (relatively earth-abundant) transition metals, scientists have devoted intensive efforts in the study of these natural machineries. Their final aim is to uncover the basic principles of metalloenzymes reactivity, and to transfer them into the design of noble-metal-free synthetic devices, such as homogeneous biomimetic catalysts, able to carry out the same transformations of the natural systems.[2] Such so-called biomimetic approach is sometimes inescapable, since the direct utilization of metalloenzymes, for the development of inexpensive and efficient technological systems, is often prohibitive.[3] Because of the elusive and intrinsically complicated chemistry of transition metals, experimental investigations generally need to be complemented by the use of computational tools (especially Quantum-Mechanical ones), which are becoming more and more popular in this research area. The main focus of our research is the QM investigation, in the Density Functional Theory (DFT) framework, of two specific iron-sulfur metalloenzymes and their related biomimetic catalysts. Both systems under study, namely [FeFe]-hydrogenases and [Mo (or V)]-nitrogenases enzymes, are intriguing for their potential application in the biofuel production and energy storage fields.[4,5] Indeed, [FeFe]-hydrogenases can reversibly store energy in the form of molecular hydrogen (starting from protons and electrons), which can be used as green and powerful fuel.[4] Nitrogenases, instead, are incredibly efficient in reducing highly inert substrates into value-added chemical. Remarkably, they can also activate CO_2 and recycle it by its direct conversion into hydrocarbons.[6] These two cases study will be taken into account to illustrate the role and the predictive power of DFT tools in the rational design of biomimetic catalysts and in the development/tuning of their reactivity and molecular properties.

1. Holm, R. H.; Solomon, E. I. *Chem. Rev.* **2014**, *114*, 3367-3368.
2. Breslow, R.; *J. Biol. Chem*. **2009**, *284,* 1337-1342.
3. Simmons, T. R.; Berggren, G.; Bacchi, M.; Fontecave, M.; Artero, V.; *Coord. Chem. Rev.* **2014**, *270*, 127-150.
4. Lubitz, W.; Ogata, H.; Rodiger, O.; Reijerse, E. *Chem. Rev*. **2014**, *114*, 4081–4148.
5. Hoffman, B. M.; Lukoyanov, D.; Yang, Z. Y.; Dean, D. R.; Seefeldt, L. C. *Chem. Rev*. **2014***, 114*, 4041–4062.
6. Hu, Y.; Lee, C. C.; Ribbe, M. W. *Dalton Trans.*, 2012, *41*, 1118–1127.

Biomolecular recognition at nanoparticle surfaces: characterization of association equilibria and mapping of binding sites

Michael Assfalg, Andrea Bortot, Serena Zanzoni, Mariapina D'Onofrio

University of Verona, Department of Biotechnology
e-mail: michael.assfalg@univr.it

The successful application of nanoparticles (NPs) in the biosciences necessitates an in-depth understanding of how they interface with biomolecules.[1] Protein–NP interactions are also of interest for the development of hybrid devices. Thus, methodologies aimed at characterizing biomolecules associating with NPs represent an indispensable tool.

Solution NMR spectroscopy is a mature technique for the investigation of biomolecular structure and intermolecular associations, however its use in protein-NP interaction studies remains highly challenging.[2] We show that noncovalent protein-NP interactions are accessible by NMR, and we describe novel approaches based on site-resolved protein signal perturbations.[3,4] Besides the mapping of binding surfaces, we also successfully applied NMR approaches to describe the dynamics of proteins adsorbed onto NPs.[5]

The developed approaches offer new opportunities for the characterization of the biomolecular corona of NPs and its role in mediating NP interactions with cells. Furthermore, our improved understanding of bio-nano interfaces should enable better control of NP biological functionality, ultimately supporting the development of NP-based drugs targeting specific biomolecular receptors.

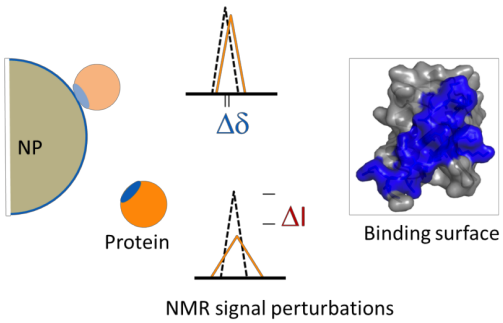


Figure 1. Mapping of binding sites on the surface of proteins interacting with nanoparticles. Perturbations of NMR signals are observed for atoms forming the contact surfaces due to exchange averaging.

1. M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. Baldelli-Bombelli, S. Laurent, *Chem. Rev.* **2011**, *111*, 5610-37;
2. M. Assfalg, L. Ragona, K. Pagano, M. D'Onofrio, S. Zanzoni, S. Tomaselli, H. Molinari, *Biochim. Biophys. Acta* **2016**, *1864*, 102-14;
3. S. Zanzoni, A. Ceccon, M. Assfalg, R. K. Singh, D. Fushman, M. D'Onofrio, *Nanoscale* **2015**, *7*, 7197-205;
4. S. Zanzoni, M. Pedroni, M. D'Onofrio, A. Speghini, M. Assfalg, *J. Am. Chem. Soc.* **2016**,138, 72-5;
5. A. Ceccon, M. Lelli, M. D'Onofrio, H. Molinari, M. Assfalg, *J. Am. Chem. Soc.* **2014**, *136*, 13158-61.

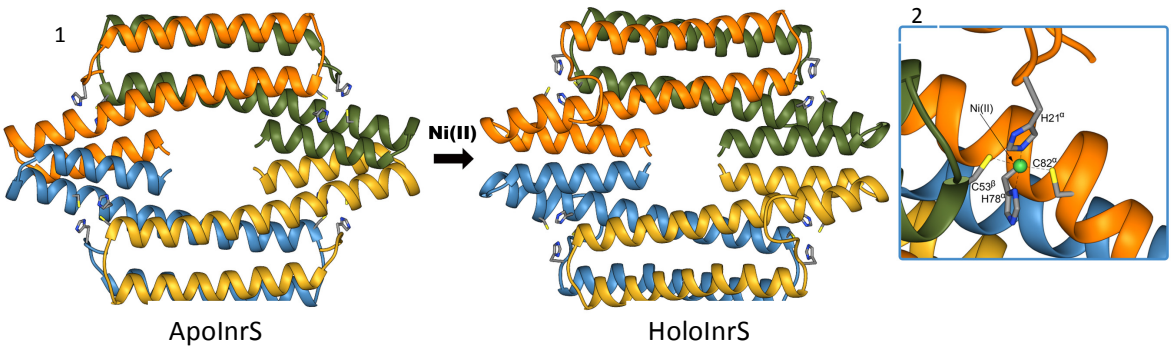
Structural studies on InrS, a Ni(II) dependent transcription factor

Elia Barchi, Stefano Ciurli, Francesco Musiani

FABIT (Unibo, Bioinorganic department)

e-mail: elia.barchi@studio.unibo.it

Transitional metal ions, such as Co2+, Ni2+ and Zi2+ are essential for the catalytic activity of a large number of enzymes involved in the metabolic processes of all the organisms. On the other hand, at high concentrations transition metal ions are toxic and require a fine regulation of their homeostasis through sensing, transport and storage systems[1]. Here we report the case of the Ni(II)-dependent transcriptional regulator InrS found in the cyanobacterium Synechocystis PCC 6803[2]. InrS repress the transcription of the nrsD gene, coding for a Ni(II)-efflux protein in the inner membrane. Our approach is divided in two parts: i) a bioinformatics approach aimed to identify the structural determinants of the metal binding and the structural differences between the apo and holo forms of InrS, and ii) a NMR approach aimed to validate and improve the structural models obtained in the previous part. Starting from the recently solved X-ray structure of InrS in the apo form we generated a structural model of the protein in the holo conformation based on the available crystal structured of other members of the same family (CroR/RcnR) [3,4,5,6]. Finally we modelled the square planar Ni(II) metal binding site of InrS by using XAS data[7] and following a well-established protocol[8].



1. conformational change between ApoInrS and HoloinrS
2. Ni^{2+}- binding site of HoloInrS

1. Musiani, F., B. Zambelli, M. Bazzani, L. Mazzei, et al. (2015). "Nickel-responsive transcriptional regulators." Metallomics **7**(9): 1305-1318.
2. Foster, A. W., R. Pernil, C. J. Patterson and N. J. Robinson (2014). "Metal specificity of cyanobacterial nickel-responsive repressor InrS: cells maintain zinc and copper below the detection threshold for InrS." Molecular Microbiology **92**(4): 797-812.
3. Foster, A.W., Pernil, R., Patterson, C.J., Scott, A.J., Palsson, L.O., Pal, R., Cummins, I., Chivers, P.T., Pohl, E., Robinson, N.J.(2017). **"**A tight tunable range for Ni(II) sensing and buffering in cells**".** Nat. Chem. Biol. **13:** 409-414
4. Liu, T., Ramesh, A., Ma, Z., Ward, S.K., Zhang, L., George, G.N., Talaat, A.M., Sacchettini, J.C., Giedroc, D.P. (2007) Nat.Chem. Biol **3**: 60-68
5. Sakamoto, K., Agari, Y., Agari, K., Kuramitsu, S., Shinkai, A. (2010) Microbiology **156**: 1993-2005
6. Chang, F.M., Coyne, H.J., Cubillas, C., Vinuesa, P., Fang, X., Ma, Z., Ma, D., Helmann, J.D., Garcia-de los Santos, A., Wang, Y.X., Dann, C.E., Giedroc, D.P. (2014) J.Biol.Chem. **289**: 19204-19217
7. Carolyn E. Carr, Andrew W. Foster, Michael J. Maroney (2017). "An XAS investigation of the nickel site structure in the transcriptional regulator InrS." J. Inorg. Biochem **177**: 352-358.
8. Carr, C. E., F. Musiani, H.-T. Huang, P. T. Chivers, et al. (2017). "Glutamate Ligation in the Ni(II)- and Co(II)-Responsive Escherichia coli Transcriptional Regulator, RcnR." Inorg Chem **56**(11): 6459-6476.

Nickel effects on human health: NDRG1 and SrnR, two proteins related to Ni^{2+} dependent tumorigenesis and infections

[1]Ylenia Beniamino

[1]Alma Mater Studiorum, Università degli studi di Bologna, Lab Biochimica Inorganica Viale Fanin 40
e-mail: ylenia.beniamino@studio.unibo.it

Nickel is the twenty-four most abundant element found in the earth's crust. This metallic compound plays fundamental biological roles for plants, bacteria, archaea and unicellular eukaryotes, catalyzing biochemical reactions in the active site of metallo-enzymes such as urease, hydrogenase, Co-dehydrogenase, acetil-CoA synthase, methyl-CoM reductase, Ni-superoxide dismutase. Despite this important role, nickel has also a poisoning potential: exposure to nickel is responsible for a variety of adverse effects on humans, such as immune reactions, respiratory and cardiovascular diseases, bacterial infections and carcinogenesis. All hazardous health effects caused by nickel exposure to human health are mediated by the interaction of nickel with macromolecules and by the formation of reactive compounds that mediate cellular damage, through changes in metabolism, inflammation, oxidative stress, cell proliferation and cell death. The aim of the present study is to determine the biochemical and biophysical properties of some proteins involved in nickel-dependent health effects.

NDRG1 (N-myc downregulated gene 1) is a human protein, whose expression is induced by nickel through the hypoxia pathway. This protein has multiple biological roles, being involved in stress response, cell cycle control, differentiation, myelin maintenance and angiogenesis. Multiple studies have revealed that the expression levels of this protein in normal tissues are lower than in tumor tissues and suggested that NDRG1 is a tumor suppressor in a variety of cancer. Proteins belonging to the NDRG family share 57-65% identity and mainly differ in the C-terminal region. In particular, NDRG1 features a C- terminal tail, with the three fold repeat GTRSRSHTSE, a possible nickel binding motif.

SrnR from *Streptomyces griseus*, a Gram positive and aerobic actinomycete that causes the subcutaneous infection of human cervicofacial mycetoma, belongs to the Ars family of transcriptional regulators. It shows a DNA binding motif and collaborates with SrnQ for the nickel-dependent repression of the gene for superoxide dismutase, sodF.

In my work, NDRG1 and SrnR have been heterologously expressed and purified from *E. coli*. Light scattering, circular dichroism and NMR will be subsequently applied to determine the structural properties of these proteins in solution.

1. *B. Zambelli, V. N. Uversky, S. Ciurli; "**Nickel impact on human health: an intrinsic disorder perspective**"; Biochimica and Biophysica Acta (2016) 1714-1731;*
2. *Rui-Hai Zhou, Koichi Kokame, Yoshitane Tsukamoto, Chikao Yutani, Hisao kato, Toshiyuki Miyata; "**Characterization of the Human NDRG gene family: A newly identified member, NDRG4, is specifically expressed in brain and heart**"; Genomics 73, 86-97 (2001);*
3. *Kim JS, Kang SO, Lee JK; "**The protein complex composed of nickel-binding srnQ and DNA binding motif-bearing srnR of Streptomyces griseus represses sodF transcription in the presence of Nickel**"; J BIo Chem 2003 May 16 ;*

Bio-based Economy: the role of industrial biotechnology

Mario Bonaccorso

Assobiotec-Federchimica/Cluster Tecnologico Nazionale della Chimica Verde SPRING (via Giovanni da Procida 11, Milano)

e-mail: m.bonaccorso@federchimica.it

Italy is a key player in the European bioeconomy due its great tradition in innovative biotech and chemical industries, excellent academic science and high-volume agricultural resources. Industrial biotechnology has been recognized as a key enabling technology to address the challenges we are facing today: in order to battle against climate change, fossil-based fuels, chemicals and materials need to be replaced; in the long run fossil resources will be depleted; international brand owners seek sustainable chemicals in applications such as packaging materials, solvents, inks, fibres, resins and paints; there is an effort to replace toxic and harmful chemicals in applications related to food or human contact.

Bioeconomy companies revolve entirely around industrial biotechnology. Their focus is the development of processes and products from renewable resources. An increasing number of chemicals and materials, like base chemicals, polymers, industrial catalysts, enzymes and detergents are already produced using biotechnology. Industrial Biotechnology has the potential to trigger a paradigm shift in the chemical industry through the step-by-step establishment of biorefinery. MacKinsey and Co. estimates that the world sales of renewable chemicals will increase from €205 billion in 2012 (9% of total chemical sales) to €344 billion (11% of total chemical sales) by 2020 with a GAGR of 8%.

In this scenario, the Italian biotech is doing very well. This industry has grown a lot in recent years. Today - according to a report published by Assobiotec, the Italian Association for the development of Biotechnology - the biotech companies operating in the country are more than 540. If we refer to the field of application of biotechnology, the area of Healthcare is the most developed, but also the industrial biotechnology sector has grown considerably in the last few years, thanks to the boost of the bioeconomy.

According to a recent analysis realized by the Research Department of Intesa Sanpaolo, one of the leading bank group in Europe, the Italian bioeconomy is worth more than 251 billion euros, with approximately 1.7 million employees.

It is clear that Italy, which in April 2017 presented its own strategy on bioeconomy, plays today an important role in the European biobased-economy, with relevant bio-based chemical companies such as Novamont, Biochemtex, Beta Renewables, Versalis (the chemical company of the oil giant ENI), but also a series of dynamic and innovative industrial biotech SMEs, which enjoy a true competitive edge, at world level, in terms of technological leadership.

The industrial biotechnology is actually a great opportunity for Italy to return to grow, reconciling economic growth, new high-skilled jobs and environmental sustainability. In a few words: a new Industrial Renaissance for Italy.

Structural studies of jaburetox, an intrinsically disordered urease-derived polypeptide

Broll, V.[1], Martinelli, A. H. S.[2], Lopes, F. C.[3], Fruttero, L. L.[3,4], Zambelli, B.[1], Salladini, E.[1], Musiani, F.[1], Dobrovolska, O.[1], Real-Guerra, R.[3], Uversky, V. N.[5], Ciurli, S.[1], Carlini, C. R[4]

[1] *Laboratory of Bioinorganic Chemistry, FaBiT, University of Bologna, Bologna, Italy.*
[2] *Department of Biophysics and Center of Biotechnology, UFRGS, Porto Alegre, Brazil.*
3 Center of Biotechnology (CBiot), UFRGS, Porto Alegre, Brazil.
[4] *Brain Institute – BRAINS – InsCer, PUCRS, Porto Alegre, Brazil.*
[5] *Morsani College of Medicine, University of South Florida, Tampa, USA*

e-mail: valbroll@gmail.com

Jaburetox (Jbtx) is an entomotoxic polypeptide released from Jack bean urease (JBU) after hydrolysis by digestive cathepsin-like enzymes. That polypeptide shows to be toxic to a broad spectrum of insects, phytopathogenic filamentous fungi and yeasts of medical importance [1]. Jbtx is known to be a monomer of 11.0 kDa [1] and its toxicity is described as a consequence of its ability to alter the permeability of membranes, hinting to a role of Jbtx-membrane interaction [2,3]. Central nervous system (CNS) of insects were pointed out as a Jbtx target [4,5]. In the last few years our group was able to determinate Jbtx as a polypeptide with low propensity to assume a stable secondary structure by nuclear magnetic resonance (NMR) spectroscopy [6]. Aiming to bring to the light Jbtx mechanism of action, structural aspects of Jbtx-membrane interaction were studied by investigating Jbtx behaviour when in presence of micelles, large unilamellar vesicles (LUVs), and bicelles, using NMR and CD spectroscopies [7]. Significant differences were observed in base of the composition of the membrane model used. The interaction with SDS micelles increases the secondary and tertiary structure content of the polypeptide, while, in the case of LUVs and bicelles, conformational changes were observed at the terminal regions, with no significant acquisition of secondary structure motifs [7]. Polypeptide slight conformation change when in presence of cell membrane models suggest that the polypeptide mechanism of action could be by a cell membrane anchor through polypeptide terminal portions. Jbtx undergoes conformational changes to achieve a more ordered structure that could facilitate its interaction with membrane-bound proteins. Consistently with this hypothesis, the presence of these membrane models decreases the ability of Jbtx to bind cellular membranes of insect nerve cord. The collected evidence from these studies implies that the biological activity of Jbtx is due to protein-phospholipid interactions.

[1]Carlini C.R. & Ligabue-Braun R.; *Toxicon* **2016**, 110, 90–109.

[2]Barros P., Stassen, H., Freitas, M.S., Carlini, C., Nascimento, M.A.C., Follmer, C.; *BBA - Proteins Proteomics* **2009**, 1794, 1848–1854.

[3]Martinelli A.H.S., Kappaun, K., Ligabue-Braun, R., Defferrari, M.S., Piovesan, A.R., Stanisçuaski, F., Demartini, D.R., Dal Belo, C.A., Almeida, C.G.M., Follmer, C., Verli, H., Carlini, C.R., Pasquali, G.; *Biochimica et Biophysica Acta (BBA) - General Subjects* **2014** 1840, 935–944.

[4]Galvani G.L., Fruttero, L.L., Coronel, M.F., Nowicki, S., Demartini, D.R., Defferrari, M.S., Postal, M., Canavoso, L.E., Carlini, C., Settembrini, B.P.; *BBA – General Subect.* **2015**, 1850, 255–262.

[5]Fruttero L.L., Moyetta, N.R., Krug, M., Broll, V., Coste Grahl, M.V., Real-Guerra, R., Stanisçuaski, F., Carlini, C.R.; *Acta Tropica* **2017**, 168, 54-63.

[6]Lopes F.C., Dobrovolska, O., Real-Guerra, R., Broll, V., Zambelli, B., Musiani, F., Uversky, V.N., Carlini, C., Ciurli, S.; *FEBS Journal* **2015**, 282, 1043–1064.

[7]Broll, V., Martinelli, A.H.S., Lopes, F.C., Fruttero, L.L., Zambelli, B., Salladini, E., Dobrovolska, O., Ciurli, S., Carlini, C.R.; *Colloids and Surfaces B: Biointerfaces* **2017**, 159, 849-860.

IMPROVING CELL-BASED ASSAYS AND WHOLE-CELL BIOSENSORS WITH BIOLUMINESCENT 3D CELL CULTURE MODELS

Maria Maddalena Calabretta[1], Antonia Lopreside[1], Elisa Michelini[1,2], Luca Cevenini[1], Aldo Roda[1,2]

[1]*Department of Chemistry "Giacomo Ciamician", University of Bologna, Via Selmi,2 , Bologna, Italy*
[2]*INBB, Istituto Nazionale di Biostrutture e Biosistemi, Viale Medaglie d'Oro 305, Roma, Italy*
e-mail: maria.calabretta2@unibo.it

Bioluminescent (BL) cell biosensors and cell-based assays based on two-dimensional (2D) cultures, represent one of the most appealing and well-established bioanalytical tools for the early stages of the drug discovery process. Conversely, thanks to the generation of the extracellular matrix and the restoring of cell-to-cell communications, 3D cell-culture models faithfully mimic *in vivo* tissue physiology. In this work a high-throughput assay for tumor necrosis factor α (TNF α) in 96 well- micro-patterned microplates is reported using a transcriptional biosensor system relying on BL 3D spheroids[1]. A new enhanced chimeric firefly luciferase mutant with improved spectral and physical properties, named PLG2, was selected as a reporter protein for engineering spheroids. PLG2 is characterized by enhanced activity compared with the *Photinus pyralis* wild-type luciferase (PpyWT) (about 35%), absence of red-shifting of bioluminescence at low pH (~6.5), and improved thermostability (24 h vs. 20 min at 37 °C). Three day-old Hek293 spheroids, transfected with a reporter construct in which the PLG2 luciferase is placed under the control of the NFkB (Nuclear Factor kB) response element, were incubated with different concentrations of tumor necrosis factor α (TNFα) (concentration range 0.1-10 ng/mL) for 5 h. The binding of TNFα to its specific endogenous receptor (TNFR) activates the intracellular inflammatory pathway, leading to PLG2 expression. Dose-response curves for TNFα were obtained with both monolayer cultures and spheroids, obtaining EC50 values of 2.6±0.4 and 3.5±0.5 ng/mL, respectively. Compared with the 2D format, a higher NFkB basal activation (4.1±0.3 fold) was found in 3D spheroids. This result is consistent with in vivo data, thus corroborating the hypothesis that spheroids provide a more physiological condition than 2D cell-based assays.

Encouraged by these results, we also evaluated the feasibility of turning these 3D cell-based assays into actual biosensors. We fabricated a cell cartridge and smartphone adaptor using a desktop 3D printer to provide a mini-darkbox and an aligned optical interface between the smartphone camera and the cell cartridge for BL signals acquisition. Moreover, multicolor bioluminescence was also implemented by exploiting two luciferases emitting at different wavelengths. We created dual-color BL spheroid-biosentinels in which a red-emitting luciferase is induced by the presence of pro-inflammatory molecules and a green-emitting reporter is constitutively expressed and used as viability control. Proof-of-principle applications are presented together with main limitations, such as those related to the limited shelf-life of cells.

1. Cevenini, L; Calabretta, M.M.; Lopreside, A.; Branchini B.R.; Southworth T.L.; Michelini, E.; Roda, A. *Photochem. Photobiol.* **2017**, *93*(2), 531-535.

In silico screening of enzymes for polycondensation using structure-based bioinformatic analysis and BioGPS descriptors

Cespugli M.[1], Ferrario V.[1], Nappi C.[1], Ebert C.[1], Gardossi L.[1]

[1] Laboratory of Applied and Computational Biocatalysis, Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Trieste, Piazzale Europa 1, 34127, Trieste, Italy

e-mail: marco.cespugli@phd.units.it

The rising demand for advanced polyesters, displaying new functional properties, has boosted the development of new biocatalyzed routes for polymer synthesis, where enzymes concretely respond to the challenge of combining benign conditions with high selectivity and efficient catalysis. Enzymes are attractive sustainable alternatives to toxic catalysts used in polycondensation, such as metal catalysts and tin in particular. [1] Moreover, they enable the synthesis of functional polyesters that are otherwise not easily accessible by using traditional chemical routes.

Enzymes add higher value to bio-based polymers by catalyzing not only their selective functionalization but also their synthesis under mild and controlled conditions. In order to understanding deeply the structural features of the enzymes used in polycondensation, a new bioinformatic methodology founded on the Unsupervised Pattern Cognition Analysis of GRID-based BioGPS descriptors (Global Positioning System in Biological Space) was employed.

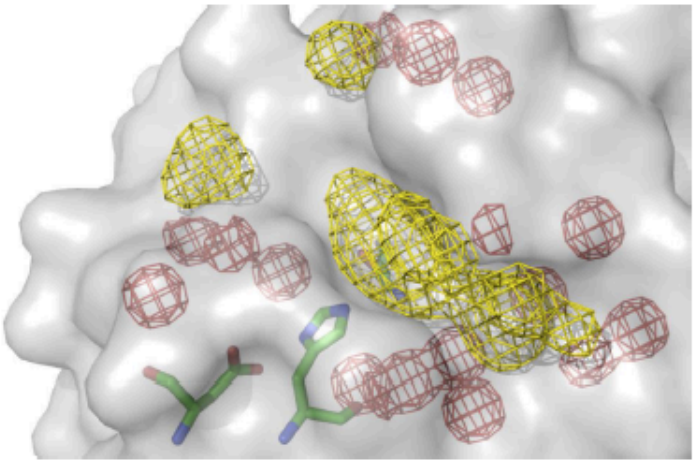


Figure 1: An example image of the differences (red) in terms of H-bond donor capabilities of the active site of Thermobidida Cellulosylitica cutinase 1 with Candida anctartica lipase 1. The area that can be occupied by H-bond acceptor moieties of the substrate is marked in yellow.

The procedure relies entirely on three-dimensional structure analysis of enzymes and does not stem from sequence or structure alignment. The BioGPS descriptors account for chemical, geometrical and physical-chemical features of enzymes and are able to describe comprehensively the active site of enzymes in terms of "pre-organized environment" able to stabilize the transition state of a given reaction. [2, 3] BioGPS analysis has been exploited in order to evaluate the suitability in polycondensation reactions of different enzymes belonging to the class of cutinases and to highlight the variable or shared structural features of the different classes of serine hydrolases processed.

NMR-based identification of anti-amyloidogenic compounds in green and roasted coffee extracts

Carlotta Ciaramelli,[1] Alessandro Palmioli,[1] Ada De Luigi,[2] Laura Colombo,[2] Gessica Sala,[3,4] Chiara Riva,[3,4] Chiara Paola Zoia,[3,4] Mario Salmona,[2] Cristina Airoldi[1,4]

[1] *Department of Biotechnologies and Biosciences, University of Milano-Bicocca, P.zza della Scienza 2, 20126 Milano, Italy.*
[2] *Department of Molecular Biochemistry and Pharmacology, IRCCS- Istituto di Ricerche Farmacologiche "Mario Negri", Via G. La Masa 19, 20156 Milano, Italy.*
[3] *School of Medicine and Surgery, University of Milano-Bicocca, Via Cadore 48, 20900 Monza, Italy.*
[4] *Milan Center of Neuroscience (NeuroMI), 20126 Milano Italy.*

e-mail: carlotta.ciaramelli@unimib.it

We developed an experimental protocol that combines NMR spectroscopy and atomic force microscopy, *in vitro* biochemical and *ex vivo* cell assays to detect anti-Aβ molecules in natural edible matrices to identify food and beverages providing regular intake of natural compounds capable of interfering with toxic amyloidogenic aggregates. In particular, we applied this approach to investigate the potential anti-amyloidogenic properties of coffee and its molecular constituents. [1]
Our data showed that green and roasted coffee extracts and their main components, 5-*O*-caffeoylquinic acid and melanoidins, can hinder Aβ aggregation and toxicity in a human neuroblastoma SH-SY5Y cell line. Coffee extracts and melanoidins also counteract hydrogen peroxide- and rotenone-induced cytotoxicity and modulate some autophagic pathways in the same cell line. In particular, the molecular interaction with a neurodegenerative amyloid oligomers model (Aβ1-42 oligomers) was demonstrated by means of STD-NMR and trNOESY-NMR spectroscopy.[2] Moreover, their antioxidant and anti-amyloidogenic activities were evaluated by cellular and biochemical assays, validating the existence of a correlation among the recognition of the molecular targets and the biological responses.
Notably, chlorogenic acids (CGAs), the most abundant family of polyphenols contained in green coffee but also in other foods from plant origin, show other beneficial biological activities, including anti-oxidant, anti-carcinogenic,[3] anti-aging activity,[4] accounting for their potential employment as nutraceuticals.

Acknowledgements. Coffee samples were kindly provided by Beyers Koffie, Belgium. We thank Fondazione Cariplo - Regione Lombardia for grant # 2015-0763.

[1]. Ciaramelli, C.; Palmioli, A.; De Luigi, A.; Colombo, L.; Sala, G.; Riva, C.; Zoia, C. P.; Salmona, M.; Airoldi, C. *Food Chemistry*. **2018**, *252*, 171–180.
2. Sironi, E.; Colombo, L.; Lompo, A.; Messa, M.; Bonanomi, M.; Regonesi, M. E.; Salmona, M.; Airoldi, C. *Chem. Eur. J.* **2014**, *20*, 13793–13800.
3. Palmioli, A.; Ciaramelli, C.; Tisi, R.; Spinelli, M.; De Sanctis, G.; Sacco, E.; Airoldi, C. *Chem. Asian J.* **2017**, *12*, 2457 – 2466.
[4] Amigoni, L.; Stuknytė, M.; Ciaramelli, C.; Magoni, C.; Bruni, I.; De Noni, I.; Airoldi, C.; Regonesi, M. E.; Palmioli, A. *J. Funct. Foods.* **2017**, *33*, 297-306.

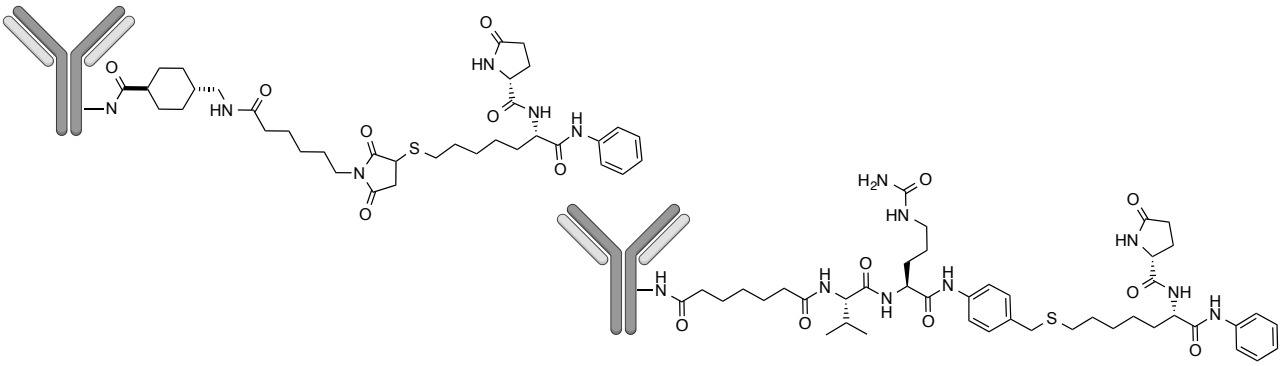
Bioconjugation Chemistry for Development of new Antibody Drug Conjugates

Elena Cini,[1] Valentina Faltoni,[2] Giuseppe Giannini,[3] Elena Petricci,[1] Laura Salvini,[4] Maurizio Taddei[1]

[1] Dip. di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena Via A. Moro 2 53100 Siena
[2]Lead Discovery Siena srl, Via Fiorentina 1, 53100 Siena,
[3]R&D Alfasigma S.p.A., Via Pontina, Km. 30.400 - 00071 Pomezia (Roma).
[4]Fondazione Toscana Life Sciences, Via Fiorentina 1, 53100 Siena.

e-mail: cinielena@libero.it

With more than 50 antibody–drug conjugates (ADCs) in clinical trials for cancer treatment, the ADC therapy opens new avenues to chemotherapy especially after recent FDA/EMA approval of ADC for treatment of Hodgkin lymphoma, systemic anaplastic large cell lymphoma, Her-2-positive metastatic breast cancer and acute lymphoblastic leukemia.[1] ADCs activity is strictly related to the chemical properties of both linkers and payloads, while the conjugation methodologies impact their homogeneity. Recently we have developed different chemical approaches to bioconjugation aimed to prepare novel ADCs that can deliver hystone deacetylase (HDAC) inhibitors for epigenetic modulation. Using both cleavable and non-cleavable linkers, different payloads were prepared and conjugated to mAbs. These products exhibited unmodified ability to recognize the target and efficient internalization into tumour cells. Animal models of human solid tumours showed high efficacy of the conjugates demonstrating that it is possible to obtain active ADCs even with not highly cytotoxic warheads, with exceptional potential of decreasing the side effects of this class of drugs. We are actively working to ascertain if the possibility to successfully bioconjugate medium/low cytotoxic drugs is limited to HDAC inhibitors or may be extended to other biologically active compounds with inconceivable consequences for therapies beyond oncology.



1. Carter, P. J.; lazar, G. A. *Nature Reviews Drug Discovery*, **2017**, doi:10.1038/nrd.2017.227.

Ligand Based Virtual Screening for Urease Inhibition

Umberto Contaldo[1], Francesco Musiani[1], Luca Mazzei[1] and Stefano Ciurli[1]

[1]*Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy.*

e-mail: umberto.contaldo@studio.unibo.it

Urease is a Ni(II)-dependent enzyme that catalyzes the hydrolysis of urea into ammonia and carbamate at a rate time 10^{15} times higher than the uncatalyzed reaction [Maroney and Ciurli 2014]. Urease is a virulence factor for ten of twelve pathogenic and antibiotic-resistant bacteria listed in 2017 by World Health Organization [W.H.O. 2017]. This observation has focused the attention of the scientific community on urease as a target for the development of new drugs for the treatment of important bacterial infections. Several known inhibitors cannot be used for clinical treatment of human patients because of their toxicity and low stability [Kosikowska and Berlicki 2011]. For these reasons here we use the Ligand Based Virtual Screening (LBVS) approach implemented in the ROCS software [Hawkins et al. 2007; OpenEye Scientific Software 2015] to enquiry large small molecules data bases in order to find new possible urease inhibitors. ROCS is a powerful virtual screening tool which can rapidly identify potentially active compounds by shape and pharmacophore comparison. In particular, we used the ZINC15 database [Sterling and Irwin 2015], composed of more than 130 million of purchasable "drug-like" compounds. The most promising molecules resulting from the virtual screening were visually inspected and a restricted subset was eventually tested *in vitro*. We found two new potential urease inactivators (1,2-dihydoxy-3-methoxybenzene and 1,4-dihydroxy-2-methoxybenzene) similar to other well-known urease inhibitors: catechol and 1,4-benzoquinone [Mazzei et al. 2016; Mazzei et al. 2017].

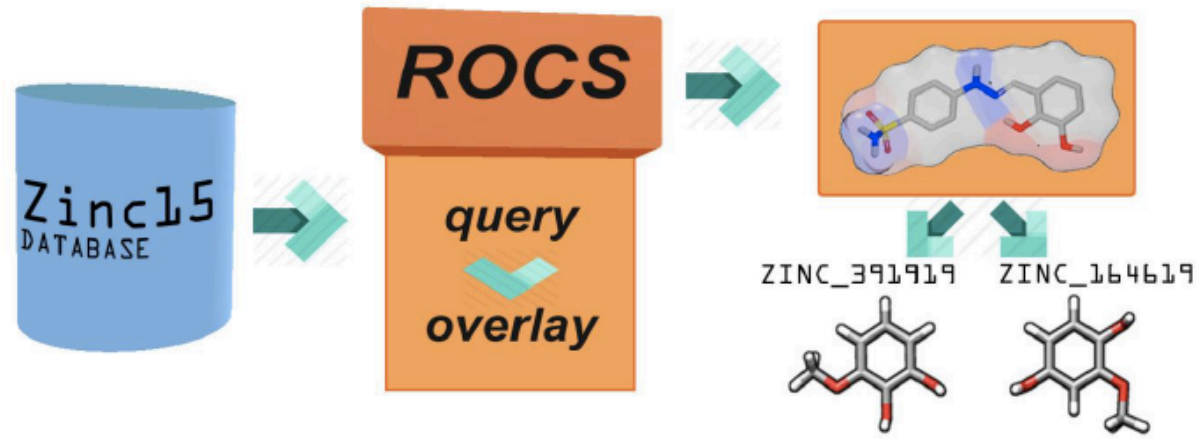


Figure 1 Flowchart of LBVS: The ZINC15 Database is processed by ROCS through by overlapping each molecule to the reference query. Among the best scoring molecules, we identified two-new potential urease inactivators: ZINC-ID ZINC_391919 and ZINC_164619), 1,2-dihydoxy-3-methoxybenzene and 1,4-dihydroxy-2-methoxybenzene, respectively.

Hawkins, P. C., A. G. Skillman and A. Nicholls (2007). "Comparison of shape-matching and docking as virtual screening tools." J Med Chem **50**(1): 74-82.

Kosikowska, P. and Ł. Berlicki (2011). "Urease inhibitors as potential drugs for gastric and urinary tract infections: a patent review." Expert Opinion on Therapeutic Patents **21**(6): 945-957.

Maroney, M. J. and S. Ciurli (2014). "Nonredox nickel enzymes." Chem. Rev. **114**(8): 4206-4228.

Design and synthesis of Peptide Nucleic Acids (PNAs) for DNA and RNA-based Therapeutic and Diagnostic Applications

Roberto Corradini, Andrea Rozzi, Saša Korom, Martina Neri

[1]*Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale-Università di Parma*
e-mail: roberto.corradini@unipr.it

Modern therapeutic strategies now encompass more and more advanced tools for the regulation of gene expression and of epigenetic factors, useful for several strategies targeting nucleic acids and their effectors. On the other hand, the ability to bind DNA in a specific manner offers the possibility of designing and produce new minimally invasive diagnostic methods, and instrumentations for detection of diseases at early stages and clinical follow-up. Our group is focused on the synthesis of Peptide nucleic acids (PNAs), polyamide analogues of nucleic acids, very effective in terms of affinity and selectivity in DNA/RNA recognition, for development on new tools for drug discovery[1] and diagnostics. Appropriate design of modified PNAs allows to improve their properties and to increase their cellular uptake.[2] New, modified versions of these molecules, in particular polyfunctional ones, are produced by our laboratory, most recently with the aid of molecular dynamics and Mmetadynamics for appropriate design (Figure),[3] and by using synthetic modular strategies.[4]

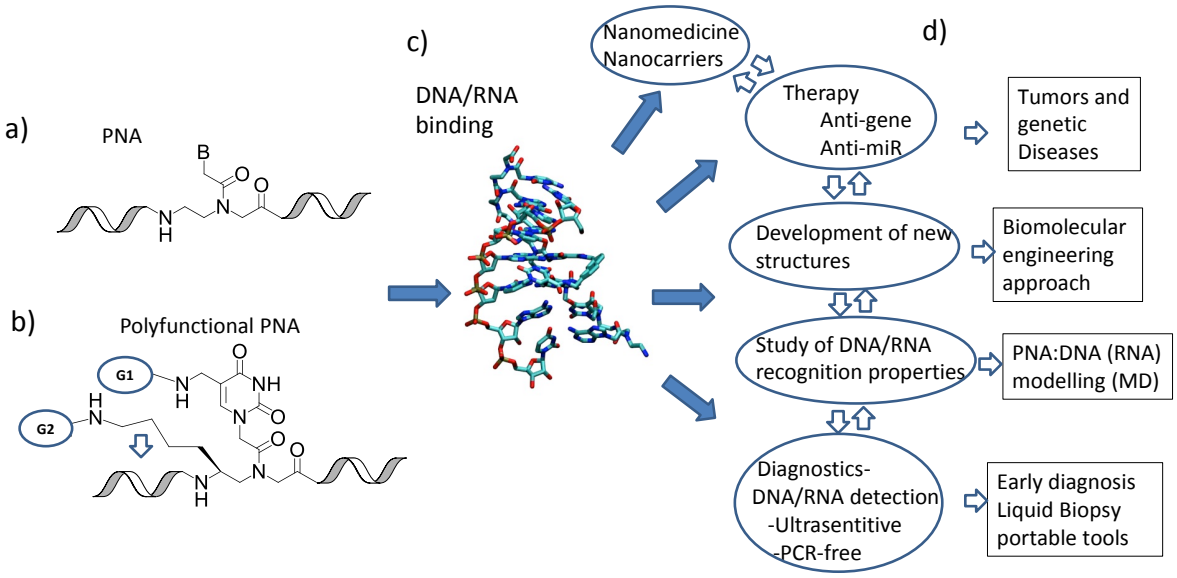


Figure. Structures of : a) PNA and b) modified PNA studied in our group; c) fields of study; d) applications

Our research is presently applied by us in collaborative works for the development of new therapeutic agents against gliomas[5] and in the treatment of cycstic fibrosis,[6] new tools in nanomedicine,[7] and new diagnostic methods for early detection of colorectal cancer.[8] Several applications for DNA detection in food have also been produced.[9]

1) Tonelli, R. et al. *Clinical Cancer Res* **2012**, *18*,796-807.
2) Manicardi, A.et al. *ChemBioChem* **2012**, *13* (9), 1327–1337.
3) Verona, M. D.; Verdolino, V.; Palazzesi, F.; Corradini, R. *Sci. Rep.* **2017**, *7*, 42799..
4) Manicardi A.; Bertucci, A.; Rozzi, A.; Corradini, R. *Org. Lett.* **2016**, *18*, 5452-5455.
5) Brognara E, et al. *Int J Oncol* **2016**; *48*:1029-1038
6) Fabbri, E. et al. *Molecules* **2018**, *23*, 71.
7) Bertucci A, et al. *Small* **2015**; *11*:5687-5695
8) H2020 EU-project ULTRAPLACAD: http://ultraplacad.eu/
9) Bertucci A, et al. *Biosens Bioelectron* **2015**; *63*:248-254

Biotech applications deriving from Quorum Sensing Studies in marine bacteria

V. Costantino

NeaNat group, TheBlueChemistryLab, Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Napoli, Italy, valeria.costantino@unina.it

e-mail: valeria.costantino@unina.it

TheBlueChemistryLab research group is involved in a programme focused on anticancer drug discovery from marine organism.[1,2] Using a new approach, based on molecular networking analysis of liquid chromatography tandem mass spectrometry (LC-MS/MS) data and Molecular Networking analysis[3] we look for novel molecules to be studied for their bioactivities in order to select novel *lead compounds* for anticancer pre-clinical screening.[1,2] More recently, we were involved in a very challenging project focused on the exploitation of the *Quorum Sensing* (QS) mechanism in marine bacteria.[4,5] In collaboration with the Steindler's group, leader in this field, we approached the study of the chemical mediators (called autoinducers) being the language that bacteria use to communicate each other. Bacteria are "*social*" organism able to collectively behave in response to these autoinducers. Processes activated by QS include, among others, biosynthesis of virulence factors and biofilm formation. In this communication, I wish to share our recent findings in this field, in light of possible applications in two field: first, biomedical, looking for a new class of antibacterial drugs, able to block the virulence of pathogen bacteria, instead of killing them; second, the use of these molecules in eco-compatible paints having antifouling properties to be used in the maritime industry.

[1] Teta R, Irollo E, Della Sala G, Pirozzi G, Mangoni A, Costantino V. Smenamides A and B, chlorinated peptide/polyketide hybrids containing a dolapyrrolidinone unit from the Caribbean sponge Smenospongia aurea. Evaluation of their role as leads in antitumor drug research. *Mar Drugs*. 2013;11(11):4451-4463. doi:10.3390/md11114451

[2] Esposito G, Teta R, Miceli R, et al. Isolation and assessment of the in vitro anti-tumor activity of smenothiazole a and B, chlorinated thiazole-containing peptide/polyketides from the Caribbean sponge, Smenospongia aurea. *Mar Drugs*. 2015;13(1):444-459. doi:10.3390/md13010444

[3] Teta R, Della Sala G, Glukhov E, et al. Combined LC-MS/MS and Molecular Networking Approach Reveals New Cyanotoxins from the 2014 Cyanobacterial Bloom in Green Lake, Seattle. *Environ Sci Technol*. 2015;49(24):14301-14310. doi:10.1021/acs.est.5b04415

[4] Costantino V, Sala G Della, Saurav K, et al. Plakofuranolactone as a quorum quenching agent from the Indonesian sponge plakortis cf. lita. *Mar Drugs*. 2017;15(3):1-12. doi:10.3390/md15030059

[5] Saurav K, Bar-Shalom R, Haber M, et al. In search of alternative antibiotic drugs: Quorum-quenching activity in sponges and their bacterial isolates. *Front Microbiol*. 2016;7(APR):1-18. doi:10.3389/fmicb.2016.00416

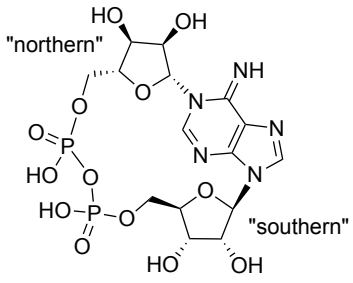
SAR studies for drug-discovery: cADPR analogues for the regulation of Ca^{2+} concentration in PC12 neuronal cells.

Stefano D'Errico,[1] Nicola Borbone,[1] Bruno Catalanotti,[1]
Valeria Costantino,[1] Gennaro Piccialli,[1] Giorgia Oliviero[2]

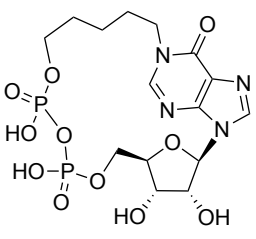
[1]Dipartimento di Farmacia, Università degli Studi di Napoli 'Federico II', via D. Montesano, 49 – 80131 Napoli
[2]Dipartimento di Biologia Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli 'Federico II', via S. Pansini, 5 – 80131 Napoli

e-mail: stefano.derrico@unina.it

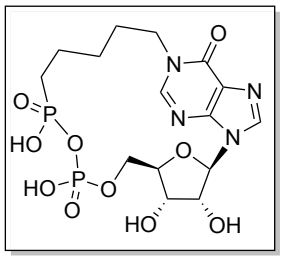
Cyclic ADP-ribose (cADPR) is a natural occurring metabolite of NAD^+ capable of mobilizing Ca^{2+} ions from intracellular stores. It was firstly isolated from sea urchin eggs extract, but it was later established that it is also produced in many other mammalian cells, including pancreatic β-cells, T-lymphocytes, smooth and cardiac muscle cells and cerebellar neurons, acting as a Ca^{2+}-mobilizing agent. For this activity, cADPR has been classified as a second messenger that, activating the ryanodine receptors of the sarcoplasmatic reticulum, is able to mobilize the calcium ions from intracellular stores. cADPR is involved in many physiological processes related to the variation of the Ca^{2+} concentration, such as the synaptic homeostasis in neurons, as well as fertilization and cellular proliferation. This cyclic nucleotide, characterized by a very labile glycosidic bond at the N1, is rapidly hydrolysed also in neutral aqueous solutions to the inactive ADP-ribose. Matsuda and co-workers[1] were the first who synthesized new analogues of the cADPR in which the adenine base was replaced by a hypoxanthine ring. This kind of modification produced the cyclic inosine diphosphate ribose (cIDPR) which proved to be stable in hydrolytic physiological conditions and showed significant Ca^{2+} mobilizing activity. A lot of modifications regarding the northern and southern ribose, as well as the purine base of cADPR, have been proposed so far. In our laboratories we have synthesized several analogues of cIDPR. In particular, the analogue with the northern ribose replaced by a pentyl chain (cpIDP) showed interesting Ca^{2+} mobilizing activity on the neuronal PC12 cell line.[2] Starting from these results, we report here the synthesis of the novel analogue cpIPP, in which the "northern" ribose of cADPR was replaced by a pentyl chain and the pyrophosphate moiety by a phophono-phosphate anhydride. The effect of the presence of the new phosphono-phosphate bridge on the intracellular Ca^{2+} release induced by cpIPP was assessed in PC12 neuronal cells.[3]



cADPR



cpIDP



cpIPP

1. Fukuoka, M.; Shuto, S.; Minakawa, N.; Ueno, Y.; Matsuda, A. *J. Org. Chem*. **2000**, *65*, 5238.
2. Mahal, A.; D'Errico, S.; Borbone, N.; Pinto, B.; Secondo, A.; Costantino, V.; Tedeschi, V.; Oliviero, G.; Piccialli, V.; Piccialli, G. *Beilstein J. Org. Chem*. **2015**, *11*, 2689.
3. D'Errico S.; Borbone, N.; Catalanotti, B.; Secondo A.; Petrozziello, T.; Piccialli, I.; Pannaccione, A.; Costantino, V.; Mayol, L.; Piccialli, G.; Oliviero, G. *Marine Drugs* submitted.

Porphyrins as versatile tool to design switches, probes and multicomponent arrays with biomolecules

Alessandro D'Urso

[1]*Dipartimento di Scienze Chimiche, Università degli Studi di Catania, viale A. Doria 6, Catania*.

e-mail: adurso@unict.it

Nature has employed porphyrins in a wide range of biological systems and for a broad range of purposes.[1] Chemists have been fascinated by the challenging syntheses of nature-inspired systems and always porphyrins represent the attractive building blocks to design supramolecular complexes. However these molecules hide manifold trouble; even if the peripheral charged groups make these macrocycles water-soluble, however porphyrins remain mainly hydrophobic molecules, preserving the well-know tendency to aggregate. This ''dichotomy'' is worthy of special attention because it plays a central role in the non-covalent syntheses of quite complex porphyrin arrays.

Here we will present how a rationalization of the spontaneous self-assembly paradigm offer tremendous potentialities to obtain a wide variety of complex systems, having specific functionality and properties. In particular we will propose an overview of the our developments in non-covalent syntheses of multi-porphyrin supramolecular species in aqueous solution: *i)* using chiral templates (biopolymers or inorganic complexes) we transfer, detect and/or amplify the matrix handedness, observing for some of such systems the chiral memory phenomenon;[2] *ii)* even we have proposed some metalloporphyrins as chiroptical probe for unusual DNA conformation (Z-DNA), demonstrating the ability of porphyrins to sense the DNA helix handedness, even in competitive environment (embedded between two portions in B-form);[3] *iii)* we investigated the potency of porphyrins as inhibitor and modulator of proteasome, which is the protein involved in many biological processes.[4] The inhibition of proteasome is apromising strategy to cure of tumors.

1. The Porphyrin Handbook, vol. 4, K.M. Kadish, K.M. Smith, R. Guilard (Eds.), Academic Press, San Diego, **2000**.

2. a) A. Mammana, A. D'Urso, R. Lauceri, R. Purrello, *J. Am. Chem. Soc.* **2007***, 129,* 8062–8063*;* b) R. Randazzo, A. Mammana, A. D'Urso, R. Lauceri, R. Purrello, *Angew. Chem.* **2008**, *120,* 10027–10030*.*

3. a) Balaz M., De Napoli M., Holmes A.E., Mammana A., Nakanishi K., Berova N., Purrello R., *Angew. Chem. Int. Ed.* **2005**, 44, 4006; b) D'Urso A., Mammana A., Balaz M., Holmes A.E., Berova N., Lauceri R., Purrello R., *J. Am. Chem. Soc.* **2009**, 131*,* 2046; c) D'Urso A., Nardis S., Pomarico G., Fragalà M.E., Paolesse R., Purrello R., *J. Am. Chem. Soc.* **2013**, 135, 8632.

4. a) A. M. Santoro, M. C. Lo Giudice, A. D'Urso, R. Lauceri, R. Purrello and D. Milardi, *J. Am. Chem. Soc.,* **2012**, 134, 10451; b) A. M. Santoro, A. Cunsolo, A. D'Urso, D. Sbardella, G. R. Tundo, C. Ciaccio, M. Coletta, D. Diana, R. Fattorusso, M. Persico, A. Di Dato, C. Fattorusso, D. Milardi, R. Purrello, *Chem Sci* **2016,** 7, 1286–1297; c) A. Di Dato, A. Cunsolo, M. Persico, A. M. Santoro, A. D'Urso, D. Milardi, R. Purrello, M. Stefanelli, R. Paolesse, G. R. Tundo, D. Sbardella, C. Fattorusso, M. Coletta, *ScIentIfIc REPORTS* **2017**, 7: 17098.

Chemical ligation approaches for protein synthesis and modification

Luca D. D'Andrea[1], Lucia De Rosa[1], Alessandra Romanelli[2]
[1]*istituto di Biostrutture e Bioimmagini, CNR, Torino.*
[2]*Dipartimento di Scienze farmaceutiche, Università di Milano, Milano.*

e-mail: luca.dandrea@cnr.it

Chemical synthesis of proteins by ligation approaches is unanimously recognized as a key strategy in protein preparation and modification, ensuring a limitless and surgically precise modification of protein covalent structure.[1] The chemical ligation approach consists in the preparation of a polypeptide chain by sequentially joining short and unprotected peptide segments. To this aim, chemical ligation exploits chemo-selective reactions between two mutually reactive functional groups placed at respectively the C- and N-terminus of two contiguous peptide segments (Fig. 1A). Depending on the functional groups involved we can obtain, at the junction site, an amide bond (native chemical ligation) or a non-peptide bond. We used such approach to prepare doubly labelled fluorescent CTPR3 proteins for folding studies,[2,3] segmentally isotopic labelled peptide/proteins for NMR studies,[4,5] and the Ig2 domain of Axl receptor in its D-enantiomeric form for mirror phage display screening (Fig. 1B).
Chemical ligation reactions also provide an excellent platform for protein conjugation/immobilization. In this context, we used an oxime-ligation reaction for the site-selective and covalent modification of the domain 2 of the Vascular Endothelial Growth Factor Receptor 1 (VEGFR1D2). The bioconjugation strategy consists in the reaction of N-terminal aldehyde derivative of the VEGFR1D2 with a probe harbouring an alkoxyamine functional group. In this way, VEGFR1D2 can be suitably modified with molecules such as a biotin, a fluorophore or a chelating unit, and could be used to detect and quantify VEGF for diagnostic purposes as well as a tool for the screening of new molecules for therapeutic applications.

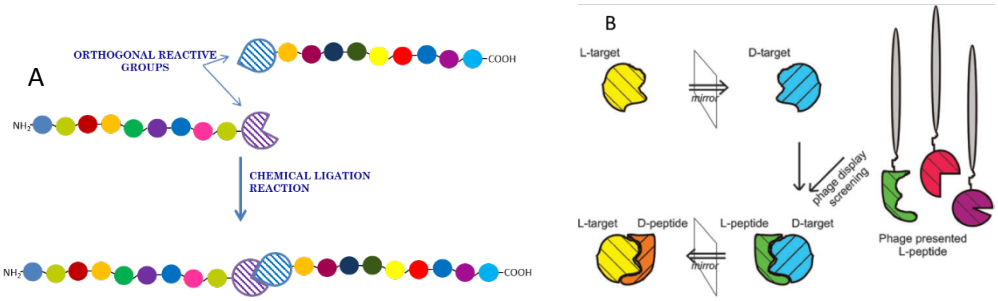


Figure 1: A) The chemical ligation reaction. B) The mirror phage display screening approach. A L-peptides will be selected to bind to an all-D protein target. This implies that the D-peptide will bind the natural L-target.[6]

1. D'Andrea, LD, Romanelli, A. (Editors) Chemical Ligation: Tools for Biomolecules Synthesis and Modification , 2017 John Wiley & Sons, Inc., Hoboken, NJ, USA
2. De Rosa L, Cortajarena AL, Romanelli A, Regan L, D'Andrea LD Site-specific protein double labeling by expressed protein ligation: applications to repeat proteins Org. Biom. Chem. 2012, 10, 273 - 280
3. Cohen SS, Riven I, Cortajarena AL, De Rosa L, D'Andrea LD, Regan L, Haran G Probing the molecular origin of native-state flexibility in repeat proteins J. Am. Chem. Soc. 2015, 137, 10367–10373
4. De Rosa, L. et al. Chemical ligation in Structural Biology in "Chemical Ligation: Tools for Biomolecules Synthesis and Modification" John Wiley & Sons, Inc., Hoboken, NJ, USA 2017, 485-515
5. Diana D, et al. Long range Trp-Trp interaction initiates the folding pathway of a pro-angiogenic β-hairpin peptide Scientific Reports 2015, 5, 16651
6. Schumacher, TN et al. Identification of D-peptide ligands through mirror image phage display.
Science 1996; 271:1854-1857

Novel peptides for biomedical applications

Luca D. D'Andrea[1], Lucia De Rosa[1], Rossella Di Stasi[1]

[1]*istituto di Biostrutture e Bioimmagini, CNR, Torino*.

e-mail: luca.dandrea@cnr.it

All biological processes are regulated by a network of protein-protein interactions whose characterization at molecular level can suggest the design of novel polypeptide binder as therapeutic and diagnostic agents. In this context, peptides, especially macrocyclic peptides, are considered ideal compounds, as they offer several advantages with respect to protein-based molecules and small organic compounds, such as high binding affinity and specificity for the target, tuneable blood clearance, low toxicity, poor immunogenicity and are easy synthesized and modified. In the last years we developed several peptides targeting the Vascular Endothelial Growth Factor Receptor (VEGFR). VEGFR and its main ligand, the VEGF, are the principal regulators of angiogenesis, a fundamental process for healing, reproduction and embryonic development whose impairment contributes to the onset, development and progression of several common and lethal human diseases, including cancer, cardiovascular disorders, retinal degeneration, and chronic inflammation. On the contrary, molecules able to activate VEGFRs may find application in therapeutic angiogenesis and in regenerative medicine. In this regard, peptides targeting VEGF receptors are useful compounds which could find application both in the diagnosis and in the treatment of a variety of VEGF-dependent diseases. Starting from the X-ray crystal structure of the complex VEGF/VEGFR (Fig. 1), we designed and characterized peptide molecules[1-6] reproducing different regions of the binding interface of the VEGF with the receptor. Combining peptide synthesis, binding studies, NMR analysis, and biological assays we demonstrated that the designed peptides were able to bind both in vitro and vivo the target receptor and appeared to be able to modulate angiogenesis.

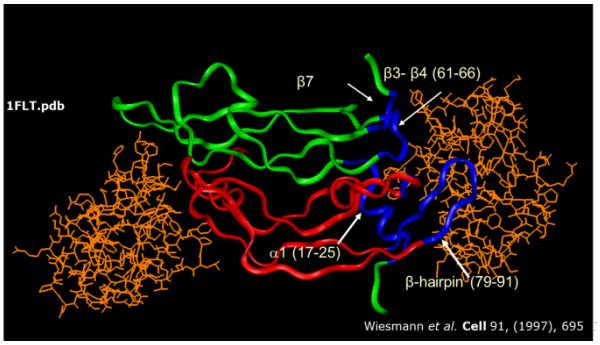


Figure 1: X-ray structure of the complex VEGF-VEGFR1. VEGF interacting regions are highlighted in blue

1. D'Andrea LD, et al., Targeting angiogenesis: structural characterization and biological properties of a de novo engineered VEGF mimicking peptide Proc. Natl. Acad. Sci. USA (2005), 102, 14215-14220

2. Basile A. et al., Characterization of a Designed Vascular Endothelial Growth Factor Receptor Antagonist Helical Peptide with Antiangiogenic Activity in Vivo J. Med. Chem. 2011, 54, 1391-1400

3. Diana D. et al., β-hairpin peptide targeting VEGF receptors: design, NMR characterization and biological activity J. Biol. Chem. 2011, 268, 41380-41691

4. De Rosa L. et al., Design, structural and biological characterization of a VEGF inhibitor b-hairpin-constrained peptide Eur. J. Med. Chem. 2014, 73, 210-216

5. De Rosa L. et al., Miniaturizing VEGF: Peptides mimicking the discontinuous VEGF receptor-binding site modulate the angiogenic response Sci. Rep. 2016, 6, 31295

6. Capasso D. et al., Unveiling a VEGF-mimetic peptide sequence in IQGAP1 protein Molecular BioSystems, 2017, 13, 1619-1629

Discovery of bioactive metabolites at NAICONS

Stefano Donadio[1]

[1]*NAICONS Srl, Viale Ortles 22/4, 20139 Milano, Italy*.

e-mail: sdonadio@naicons.com

Natural products represent a major source of approved drugs and still play an important role in supplying chemical diversity for applications in human and veterinary medicine, and for agrochemicals. This source of diversity is particularly important for antibiotics, since most of the approved drugs are of microbial origin, or derivatives thereof, while alternative approaches to antibiotic discovery have not proven as productive. In the course of the presentation, I will provide a brief overview on NAICONS' history, its technology platform, its business model and the approaches to discovery new bioactive molecules. I will also report some recent examples on new chemical classes of bioactive molecules and how they were discovered.

PNA-functionalized adenoviral vectors as new tool for gene modulation in anti-cancer treatment

Andrea P. Falanga[1], Nicola Borbone[1], Stefano D'Errico[1], Brunella Pinto[1], Maria Marzano[1], Vincenzo Cerullo[2], Gennaro Piccialli[1] and Giorgia Oliviero[3].

[1] *University of Naples "Federico II", Department of Pharmacy, Via D. Montesano 49, 80131 - Naples, Italy.*

[2] *University of Helsinki, Faculty of Pharmacy, Biocenter 2, Viikinkaari 5E, 00790 – Helsinki, Finland.*

[3] *University of Naples "Federico II", Department of Molecular Medicine and Medicinal Biotechnology, Via Pansini 5, 80131 - Naples, Italy.*

e-mail: andreapatrizia.falanga@unina.it

Guanine-rich DNA sequences fold in quadruplex structures under physiological concentrations of K+. G-quadruplexes are found throughout significative regions of human genome, for example promoters of oncogenes, such as Bcl-2. The abnormal overexpression of Bcl-2 protein is associated with many human tumours. Human Bcl-2 gene contains GC-rich regions upstream of the P1 promoter that are involved in the regulation of Bcl-2 gene expression. Several oligonucleotides (ON) and ON analogues are employed as tools to counteract the expression of this oncogene. Among these the Peptide Nucleic Acids (PNAs) are the most promising. PNAs are mimic of DNA in which sugar-phosphate back-bonds are replaced by a ethylamine glycine moiety, due these characteristic they can form, with DNA target, some structures more stable than the natural DNA/DNA complexes (1-5). To down-regulate anti-apoptotic Bcl-2 oncoproteins, here we propose an anti-gene approach based on PNA oligomers, allowing to target the bcl2G4-1 DNA sequence. Structural interactions towards the DNA target have been investigated by chemical-physical techniques. PNAs can interact with the Bcl-2 quadruplex target, conversely are not able to recognize the duplex counterpart as shown by Circular Dichroism (CD), CD-melting and PAGE studies. The main drawback of PNAs is the inability to cross phospholipid bilayers spontaneously. However PNAs are able to elicit their action only when enter cell nuclei. Fluorescence microscopy demonstrated that the suitably-FITC-labeled oligomers, specifically reach tumor cell nuclei once inside cells after electroporation.
Starting from these results in order to overcome this limit we developed a PNA delivery system for on demand administration. In this work, exploiting the ability of Adenovirus (Ad) to infect and kill tumor cells selectively (6), PNA-functionalized Ad for tumor targeting-mediated release have been successfully synthesized by tethering the resulting adduct to the surface of viral particles. We demonstrated that the conjugation to oncolytic adenovirus allow to carry the PNA inside cell nuclei working as a nuclear targeting signal, inducing a synergistic effect of both Ad and PNA in cytotoxic activity towards tumor cells. This approach holds promise to improve a site specific and safe chemotherapy, reducing the unwanted toxicity to healthy tissues and organs.

References

1. Brown, S.C., Thomson, S.A., Veal, J.M. and Davis, D.G., *Science*, 265 (1994) 777.
2. Rasmussen, H., Kastrup, J.S., Nielsen, J.N., Nielsen, J.M. and Nielsen, P.E., *Nat. Struct. Biol.*, 4 (1997) 98.
3. Leijon, M. et al., *Biochemistry*, 33 (1994) 9820.
4. Eriksson, M. and Nielsen, P.E., *Nat. Struct. Biol.,* 3, (1996) 410.
5. Amato, B. Pagano, N. Borbone, G. Oliviero, E. De Pauw, S. D. Errico, V. Piccialli, M. Varra, C. Giancola, G. Piccialli, L. Mayol, *Bioconjugate chem*, (2011) pp. 654–663,.
6. Cerullo V., Diaconu, V Romano, M Hirvinen, M Ugolini. *Molecular Therapy*, (2012): 2076-2086.

Unravelling the shape and structural assembly of photosynthetic GAPDH complexes from *Arabidopsis thaliana*

Simona Fermani[1], Libero Gurrieri[2], Alessandra Del Giudice[3], Nicolae Viorel Pavel[3], Giuseppe Falini[1], Roberto Marotta[4], Paolo Trost[2], Francesca Sparla[2]

[1]*Department of Chemistry "G. Ciamician," University of Bologna, Bologna, Italy*.
[2]*Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy*.
[3]*Department of Chemistry, University of Rome 'Sapienza', Rome, Italy*
[4]*Italian Institute of Technology IIT, Genoa, Italy*

e-mail: simona.fermani@unibo.it

The study of the tridimensional structure of biological macromolecules is a starting point to understand their structure–function relationships. To date, X-ray crystallography is the most used method and provides the most detailed information in the structural study of biological macromolecules. In the last decade, other techniques as small-angle X-ray scattering (SAXS) or electron microscopy in cryogenic conditions (Cryo-EM) have become powerful tools to structurally characterize biological macromolecules and macromolecular complexes in solution.

A combination of these different structural techniques has been used to characterize a biological system composed of a crucial enzyme of the Calvin-Benson (CB) cycle, a metabolism tightly linked to the light reactions of photosynthesis by which oxygenic photosynthetic organisms produce sugars from light. [1] Several enzymes of the CB cycle, are regulated by different mechanisms, including the formation of disulphide bonds and protein complexes.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the single reducing step of the CB cycle by consuming most of the NADPH and ATP produced by the light phase of photosynthesis. GAPDH (A_4 isoform) and phosphoribulokinase (PRK) a second enzyme of CB cycle, form with the regulatory protein CP12 a supramolecular complex having the formula $(GAPDH\text{-}CP12\text{-}PRK)_2$, in which both enzyme activities are transiently inhibited during the night. [2] The structure of the binary complex GAPDH/CP12 from *Arabidopsis thaliana*, was solved by X-ray diffraction showing that complex formation depend on the C-terminal disulfide of CP12. [3] SAXS analysis has been performed on GAPDH/CP12/PRK complex and its components. [4] Moreover, the main plant GAPDH isoform (A_2B_2) forms stable and inactive hexadecamers (A_8B_8) when it binds NAD(H) under oxidizing conditions. The crystal structure of the oxidized A_2B_2 has been determined, [5] while the low resolution structure determination of the complex A_8B_8 by Cryo-EM is now in progress.

1. Michelet L., Zaffagnini M., Morisse S., Sparla F., Pérez-Pérez M. E., Francia F., Danon A., Marchand C. H., Fermani S., Trost P., Lemaire S.D. *Front. Plant Sci.* **2013**, doi: 10.3389/fpls.2013.00470.
2. Marri L., Trost P., Trivelli X., Gonnelli L., Pupillo P., Sparla F. *J. Biol. Chem*. **2008**, *283*, 1831-1838.
3. Fermani S., Trivelli X., Sparla F., Thumiger A., Calvaresi M., Marri L., Falini G., Zerbetto F., Trost P. *J. Biol. Chem.* **2012**, *287*, 21372-21383.
4. Fermani S., Sparla F., Falini G., Martelli L., Casadio R., Pupillo P., Ripamonti A., Trost P., *Proc. Nat. Acad. Sci USA* **2007**,*104*, 11109-11114.

Alcohols and amines bio-oxidation using Laccase-mediator system

Paola Galletti[1], Daria Giacomini[1]

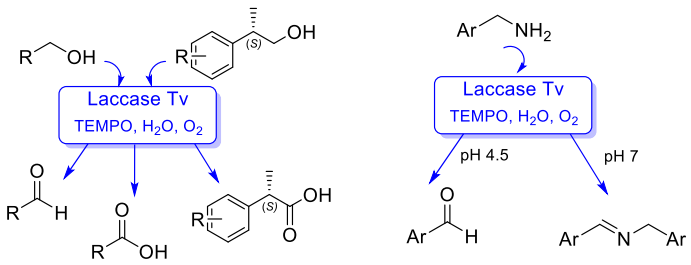
Department of Chemistry "G. Ciamician", University of Bologna, Via Selmi 2, 40126 Bologna, Italy

e-mail: paola.galletti@unibo.it

Application of biocatalysis to develop more benign and selective redox processes is becoming pivotal. The use of enzymes as catalysts meets the need of modern chemistry for more selective and clean manufacturing technologies, reducing management of hazardous chemicals and waste, thus realizing more sustainable and environmental friendly processes. Bio-oxidations have the added value of high levels of selectivity (regio- chemo- and stereo-) reliable even to fine chemicals with complex structures and possessing oxidation sensitive functional groups.

Laccases (EC 1.10.3.2) belong to the multi-copper family of oxidases. These enzymes contain four copper centers per protein molecule and catalyse the oxidation of electron rich aromatic substrates, usually phenols or aromatic amines using oxygen as the electron acceptor. Being water the only by-product formed, in principle they are ideal catalysts for sustainable chemical and technological processes. Although the natural substrates of laccases are the phenolic residues of lignin, the inclusion of appropriate mediators in the so called laccase-mediator system (LMS), makes accessible the oxidation of non-phenolic substrates. Commonly, laccases oxidize secondary alcohols to ketones and primary alcohols to the corresponding aldehydes whereas the overextended oxidation of primary alcohols to carboxylic acid is infrequent. Concerning the oxidation of amines, very few applications of Laccases were reported, mainly on anilines.

We recently report developments in the chemo-enzymatic oxidation by commercial Laccase from Trametes versicolor (TvL) of some primary alcohols and benzylamines. Primary alcohols were selectively converted to aldehydes or to carboxylic acids. The range of applicability of the bio-oxidation to carboxylic acids was widened applying the optimized protocol to the oxidation of 2-arylpropanols (Profenols) to the corresponding 2-arylpropionic acids (Profens), in high yields and with complete retention of configuration.[1] In the oxidation of benzylamines, we found that, depending on the reaction conditions, the bio-oxidation could be selectively driven to give the corresponding aldehydes or imines in good yields.[2]



1. Galletti, P.; Pori, M., Funiciello, F.; Soldati, R., Ballardini, A.; Giacomini, D. *ChemSusChem* **2104**, *7*, 2684-2689.
2. Galletti, P.; Funiciello, F.; Soldati, R., Giacomini, D. *Advances Synthesis & Catalysis* **2015**, *357*, 1840–1848

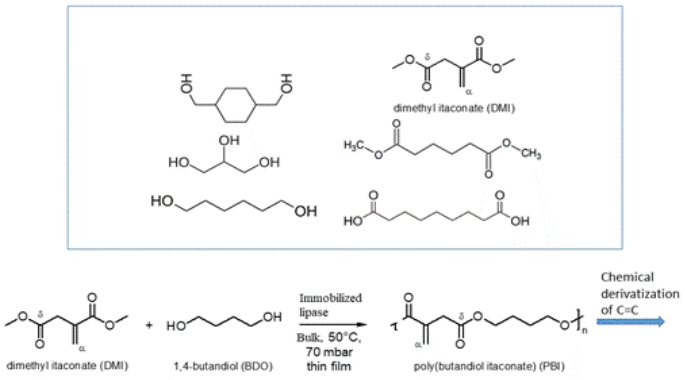
Chemo-enzymatic valorization of bio-based building blocks for the synthesis of functionalyzed polyesters

Lucia Gardossi[1], Cynthia Ebert, Marco Cespugli, Viola Cutifani, Alice Guarneri

[1]*Laboratorio di biocatalisi Applicata e Computazionale, Dipartimenti di Scienze Chimiche e Farmaceutiche, Università degli Studi di Trieste, Piazzale Europa 1, 34127, Trieste*

e-mail: gardossi@units.it

The unique selectivity of enzymes along with their anazing catalytic activity under mild conditions constitute a powerful tool for transforming bio-based building blocks into renewable polyesters[1]. We have developed an integrated approach for the study of enzymatic polycondensation of a number of functionalized bio-based monomers (Scheme 1), by combining experimental and computational investigations.



Scheme 1: Bio-based monomers used in enzymatic polycondensation and an example of enzymatic synthesis of polyesters of itaconic acid

New structure-based bioinformatics methods led to the identification of hydrolases endowed with desired catalytic properties. Analysis of structural features of enzymes guided the selection of optimal monomers and the development of tailored immobilized biocatalysts. Renewable immobilization carriers were developed for making the polycondensation of bio-based monomers truly renewable. The use of lipases under mild conditions allowed for the synthesis of polyesters of itaconic acid bearing the C=C intact, which is not achievable through classical chemical polycondensation.[2,3,4] The resulting polyesters were investigated for their ability to be chemically modified at the C=C bond *via* Michael addition.

1. Pellis, A., Ferrario V., Cespugli M., Corici L., Zartl B., Herrero Acero E., Ebert C., Guebitz, Ebert C., Guebitz G.M., Gardossi L.. "Fully renewable polyesters *via* polycondensation catalyzed by *Thermobifida cellulosilytica* cutinase 1: an integrated approach". *Green Chem.*, **2017**, ***19***, 490-502.

2. Corici L., Ferrario V., Pellis A., Ebert C., Lotteria S., Cantone S., Voinovich D. and L. Gardossi, "Large scale applications of immobilized enzymes call for sustainable and inexpensive solutions: rice husk as renewable alternative to fossil-based organic resins." *RSC Adv.*, **2016**, *6*, 63256-63270.

3.Ferrario V., Ebert C., Cantone S., Gardossi L., "Understanding potentials and restrictions of solvent-free enzymatic polycondensation of itaconic acid: an experimental and computational analysis". *Adv. Synth. Cat.*, ***2015***, *357*, 1763-1774.

4.Pellis A., Corici L., L. Sinigoi, N. D'Amelio, D. Fattor, V. Ferrario, C. Ebert, L. Gardossi, "Towards feasible and scalable solvent-free enzymatic polycondensations: integrating robust biocatalysts with thin film reactions". *Green Chem.*, **2015**, *17,* 1756-1766

Biomaterials for tissue engineering: when chemistry meets biotechnologies

Roberto Guizzardi[1], Ivan Andreosso[2], Luca Vaghi[2], Marcello Marelli[3], Annj Zamumer[4], Monica Dettin[4], Paola Brun,[5]Antonio Papagni[2], Laura Cipolla[1]

[1]*Dipartimento di Biotecnologie e Bioscienze, Università degli studi di Milano-Bicocca, Milano.*
[2]*Dipartimento di Scienze dei Materiali, Università degli studi di Milano-Bicocca, Milano.*
[3]*CNR, Istituto di Scienze e Tecnologie Molecolar (ISTM), Milano.*
[4]*Dipartimento di ingegneria Industriale, Università degli Studi di Padova, Padova.*
[5] *Dipartimento di Medicina Molecolare, Università degli Studi di Padova, Padova.*
e-mail: r.guizzardi@campus.unimib.it

In the last two decades regenerative medicine has gained increasing interest as a possible route to contrast organ failure and tissue injury. Key players in regenerative medicine strategies are biomaterials, that are designed and bio-activated through innovative chemical strategies. Among others, biomaterials can be based on natural polymers, presenting the advantage of possessing intrinsic biological properties suitable for cellular adhesion, proliferation and eventually differentiation.[1] Protein such as collagen and elastin, or polysaccharides (alginates, hyaluronic acid, chitosan) are emerging as natural biomaterials of clinical interest. In this context we will present two examples of biomaterial bio-activation strategies.

1) 2D collagen scaffolds functionalization in order to stimulate angiogenesis through HVP and Tb4, peptides considered for their role in angiogenesis development[2].

2) 3D nanostructured hydrogels obtained by innovative cross-linking chemoselective chemistry We explored tyrosine chemistry and an innovative crosslinking agent in order to obtain an extracellular 3D-matrix-like microenvironment.[3]

[1] Lutolf, M. P.; Hubbell, J. a. *Nat. Biotechnol.* **2005**, 23 (1), 47–55.
[2]Deborah, P. et al. *The FASEB journal* 17.14, **2003**, 17.17, 2103-2105.
[3]Yang, C. et al. *BioDrugs*. **2004**, 18, 103–119.

Chemistry and biotechnology for immunodiagnostics: a tight synergy for innovation

Paolo Ingallinella

DiaSorin Research Center, via Lepetit 34, 21040 Gerenzano (VA)

e-mail: paolo.ingallinella@diasorin.it

Immunoassays are powerful and well established analytical methods, representing remarkable examples of applied biotechnology and biochemistry. In the last decades, the core components of immunoassays have undergone several significant improvements bringing ever more sophisticated assays onto the market, which have had a profound influence on medicine, spanning basically all the clinical areas and achieving high levels of specificity and sensitivity routinely. In particular, chemiluminescence methods have become established in routine clinical analysis, such those marketed for use with DiaSorin full automated analyzers, LIAISON® (http://diasorin.com).

The innovation in the immunodiagnostic field is a result of integrated efforts of several scientific disciplines, like biotechnology, chemistry, biochemistry, engineering. The immunoassay research and development is currently very active and in continuous evolution to meet the most challenging analytical targets with the highest assay performances. Despite the diversity of technical approaches, the immunoassay methods share several basic key components representing the main research fields for innovation: (i) antibodies and proteins, the most critical reagents, having major contribution to assay specificities and overall analytical performance, (ii) conjugation methods, for protein labelling or solid phase immobilization, as well as for generation of multi-functional biomolecular reagents, (iii) signal technology, for detection and quantification of the target analytes, with direct impact on assay sensitivities. An overview of the main technological platforms used for generation of high-quality bioreagents for immunodiagnostics will be provided.

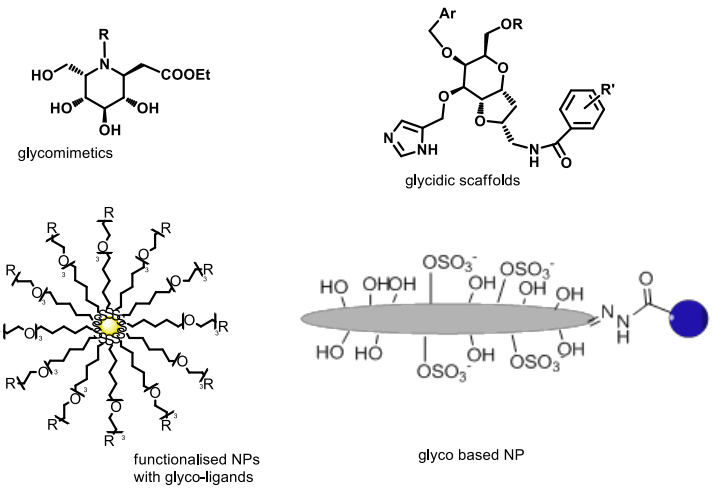
Glycotools for biotecnological applications

Barbara La Ferla, Laura Cipolla, Cristina Airoldi.

[1]University of Milano-Bicocca, Dep. of Biotechnology and Bioscience, Piazza della Scienza 2, I-20126, Milan, Italy;

barbara.laferla@unimib.it

Carbohydrates play a crucial roles in a plethora of biological, physiological and pathological process. Carbohydrate-carbohydrate and carbohydrate-protein interactions mediate the cellular interaction with the environment and trigger many events among which, bacteria and virus adhesion, fertilization, cell migration and tumor metastasis. Defective carbohydrate processing enzymes are at the basis of different pathologies such as the lysosomal storage diseases, and altered protein glycosylation may have severe consequences on protein functions. For these reasons the development of novel glycotools may represent a way to study and better comprehend physiological and pathological processes based on carbohydrate, may become novel drug candidates to treat carbohydrate based pathologies, may exploit carbohydrate based interaction to target specific tissue-organs-organism and may trigger tissue-organ regeneration.
In this context, our laboratories are focused on the generation of different kinds of glycotools:
1) enzyme inhibitors of carbohydrate processing enzymes: we are developing selective inhibitors/modulators of different enzymes for the generation of potential drugs; 2) glycidic scaffolds: we exploit sugars for their physico-chemical properties as scaffolds for pharmacophoric entities; 3) glycodendrons and glyco-decorated nanoparticles (NPs): multivalent presentations of sugars for their receptors; 4) glyco-based nanoparticles NPs for tissue targeting; 5) glyco-functionalized materials for tissue regeneration; 6) glycobased biosensor systems.



References

4. C. Airoldi, et al. *Chem Commun* 47 (2011) 10266.
5. C. Airoldi, et al. *Eur. J Med. Chem* 85 (2014) 43-50.
6. L. Colombo, et al. *Biomacromolecules* (2015), 16, 2862-2871.
7. G. D'Orazio et al. *J. Material Chem. B* (2017), 5, 7018-7020.
8. B. La Ferla, et al. *Electroanalysis*, 2018, 30, in press.
9. L. Russo, L. Cipolla *Chem. Eur. J.* (2016,) 22, 13380-8

Biochemical and structural studies on the inhibition of urease, a nickel-dependent virulence factor

Mazzei, L.[1], Musiani, F.[1], Lente, G.[2], Palombo, M.[1], Cianci, M.[3], Benini, S.[4], Ciurli, S.[1]

[a] *Dept. of Pharmacy and Biotechnology, University of Bologna (Italy);*
[b] *Dept. of Inorganic and Analytical Chemistry, University of Debrecen (Hungary);*
[c] *Dept. of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche (Italy);*
[d] *Faculty of Science and Technology, Free University of Bolzano (Italy)*

e-mail: luca.mazzei2@unibo.it

Urease is a Ni(II)-dependent enzyme that catalyzes the hydrolysis of urea to give ammonia and CO_2, determining an overall pH increase and causing negative effects for human health as well as agriculture.[1] Hence, the scientific community has devoted intense efforts in the last decades for the development of efficient inhibitors of urease able to counteract its negative effects.[1]

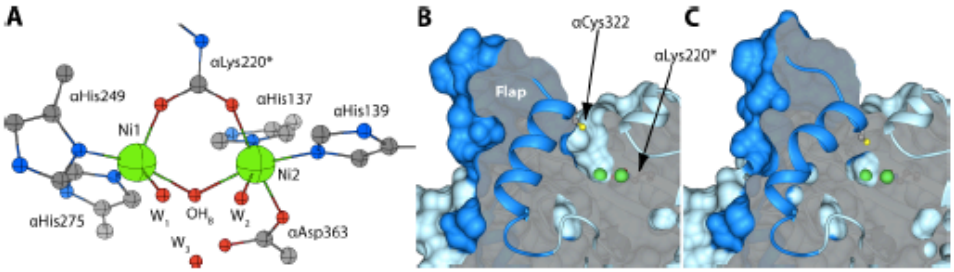


Figure 1. (A) Coordination environment of the Ni(II) ions in the active site of urease from *Sporosarcina pasteurii* (SPU). (B and C) Longitudinal section of the open (B) and closed (C) conformations of the flexible flap in SPU.

In this work, a combination of kinetic experiments and X-ray protein crystallography has been applied to the urease system in order to determine the inhibition mode of several known urease inhibitors: i) fluoride, ii) sulphite, iii) 1,4-benzoquinone (BQ), iv) catechol (CAT) and v) N-butylthiophosphotriamide (NBPT). Both fluoride and sulphite show a pH-dependent inhibition on urease, directly binding to the two Ni(II) ions in the enzyme active site.[2,3] Unlike the previous cases, BQ and CAT act as time-dependent urease inhibitors covalently binding to a conserved cysteine residue located on a flexible flap that controls the access of the substrate to the active site cavity.[4,5] NBPT, a commercial nitrogen stabilizer extensively used in agriculture, acts as a slow-binding inhibitor of urease. In particular, it directly interacts with the nickel ions in the urease active site, undergoing an *in situ* hydrolysis that generates a tetrahedral moiety blocking the active site and precluding the enzyme from further substrate hydrolysis.[6]
All the results shown in this work will be useful to develop, through a structure-based drug design procedure, novel and more efficient urease inhibitors.

1. Mazzei, L.; Musiani, F.; Ciurli, S. - Urease, in "*The Biological Chemistry of Nickel*", **2017**, 60-97.
2. Benini, S.; Cianci, M.; Mazzei, L.; Ciurli, S. - *J. Biol. Inorg. Chem.* **2014**, *19*, 1243-1261.
3. Mazzei, L.; Cianci, M.; Benini, S.; Musiani, F.; Bertini, L.; Ciurli, S. - *J. Inorg. Biochem.* **2016**, *154*, 42-49.
4. Mazzei, L.; Cianci, M.; Musiani, F.; Ciurli, S. - *Dalton Trans.* **2016**, *45*, 5455-5459.
5. Mazzei, L.; Cianci, M.; Musiani, F.; Lente, G.; Palombo, M.; Ciurli, S. - *J. Inorg. Biochem.* **2017**, *166*, 182-189.
6. Mazzei, L.; Cianci, M.; Musiani, F.; Ciurli, S. - *Biochemistry* **2017**, *56*, 5391-5404.

Raman imaging as tool for the label free characterization of cells and tissue

Morasso Carlo[1], Renzo Vanna[1], Francesca Piccotti[1], Serena Mazzucchelli[2], Marta Truffi[2], Fabio Corsi[1-2]

[1]*Nanomedicine and molecular imaging lab. ICS Maugeri S.p.A SB (Via Maugeri 10, 27100 Pavia Italia).*
[2] *Dipartimento di Scienze Biomediche e Cliniche "Luigi Sacco", Università degli Studi di Milano, (via Giovanni Battista Grassi, 74, 20157 Milano, Italia).*

e-mail: carlo.morasso@icsmaugeri.it

Raman is a vibrational spectroscopy based on the inelastic scattering of light able to provide detailed information on the chemical composition of the sample analysed virtually without any sample preparation and in label-free. While traditionally Raman spectroscopy has been mostly used by pharma industries for the characterization of raw materials, more recently, thanks to the advancement of instrumentations and thanks to the use of novel chemometric approaches, Raman is becoming a powerful tool for the characterization of biological samples with potential clinical applications. [1]

Here we report some examples resulting from the coupling of confocal Raman microscopy and multivariate analysis for the label-free monitoring and characterization of cells and tissues. Starting from the mentioned advantages, we demonstrated the potentialities of Raman imaging for the monitoring of nanodrugs internalization in cultured cells[2,3] thus permitting to verify the nanodrugs activity and its intracellular localization and eventual degradation processes. In addition, a similar approach has been used for the study of breast cancer tissue for diagnostic purposes. In particular, in figure 1, we show a breast cancer frozen slice on glass analysed without the use of any specific marker or staining procedure (figure 1).

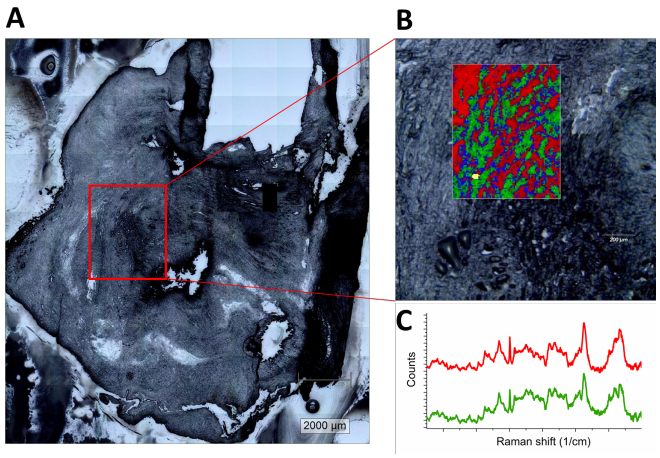


Figure 1: False-colour Raman image (B) derived from the multivariate analysis of a frozen slice of breast cancer tissue (A). The two spectra represent two of the different cellular subtypes which compose the tumour lesion (C).

1. Smith, R. et al. *Analyst.* **2016***,* 141(12), 3590-3600.
2. Verderio, P. et al. *Mol Pharm.* **2014**,11(8), 2864-2875.
3. Pandolfi, L. et al. *Biomacromolecules*. **2017** 18(10), 3318-3330.

How proteins handle Ni(II) ions: the case of urease activation

Musiani F.;[1] Masetti M.;[2] Gioia D.;[2] Bernetti M.;[2,3] Falchi F.;[3] Recanatini M.;[2] Cavalli A.;[2,3] Ciurli S.[1]

[1]*Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy.*

[2]*Laboratory of Computational Medicinal Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy.*

[3]D3 Compunet, Istituto Italiano di Tecnologia, Genova, Italy.

e-mail: francesco.musiani@unibo.it

All living beings need transition metal ions as micronutrients. Their essentiality, coupled with their limited environmental availability and toxicity, has stimulated all life forms to develop mechanisms for selective accumulation and utilization.[1] One of the most relevant example is the human pathogen *Helicobacter pylori* (*Hp*). This bacterium takes advantage of the activity of urease, a Ni(II)-dependent enzyme, to survive in the acidic conditions found in the gastric environment.[2] Urease catalyzes urea degradation to ammonia and carbonate, causing pH to increase to values suitable for bacterial survival.[3] In vivo, urease is synthesized as an inactive apoenzyme, and four accessory proteins (UreD, UreF, UreG, and UreE) are involved in a multistep process leading to the Ni(II)-loaded active enzyme.[4] The recently published crystal structure of the apo *Hp*($UreG_2$-$UreF_2$-$UreD_2$) complex[5] shows a central cavity at the interface between UreF and UreG that contains a cluster of water molecules, connected to two similar and symmetric tunnels going along the $UreF_2$-$UreD_2$ complex from the central cavity to the external edge of UreD. We theorize that Ni(II) ions can proceed through these tunnels to eventually reach the urease active site.[5] To test this hypothesis, extensive molecular dynamics simulations were carried out, and the geometrical and dynamical features of these tunnels were established.[6] In particular, these tunnels are stable on the sub-microsecond timescales, and they are able to diffuse water molecules. Concomitantly, a systematic parameterization of the Ni(II) ion was carried out to be used with classical potentials.[7] By adopting a multi-site representation, we investigated the combinations of parameters returning the coordination number, ion-water distance, and free energy of hydration in optimal agreement with experimental data. The best models were finally challenged for their ability to reproduce the ion-water exchange rates. Taken together, these studies will be instrumental to explicitly investigate the Ni(II) trafficking inside the protein tunnels using atomistic simulations.

1. Finney, L.A. and O'Halloran, T.V. *Science* **2003**, 300, 931-936.
2. Slonczewski, J.L.; Fujisawa, M.; Dopson, M.; Krulwich, T.A. *Adv. Microb. Physiol.* **2009**, *55*, 1-79.
3. Maroney M.J. and Ciurli S. *Chem. Rev.* **2014**, *114*, 4206-4228.
4. Zambelli, B.; Musiani, F.; Benini, S.; Ciurli S. *Acc. Chem. Res.* **2011**, *44*, 520-530.
5. Zambelli, B.; Berardi, A.; Martin-Diaconescu, V.; Mazzei, L.; Musiani, F.; Maroney M.J.; Ciurli S. *J. Biol. Inorg. Chem.* **2014** *19*, 319-334.
6. Musiani, F.; Gioia, D.; Masetti, M.; Falchi, F.; Cavalli, A.; Recanatini, M.; Ciurli, S. *J. Chem. Theory Comput.* **2017**, *13*, 2322-2331.
7. Masetti, M.; Musiani, F.; Bernetti, M.; Falchi, F.; Cavalli, A.; Ciurli, S; Recanatini M. *J. Comput. Chem.* **2017**, 38, 1834-1843.

Targeting GRPR expressing tumours: NMR structural insights of bombesin interaction and synthesis of potential mimetics.

Alessandro Palmioli[1], Cecilia Ceresa[2], Marcella Rocchetti[1], Barbara La Ferla[1] Cristina Airoldi[1]

[1] *Dept of Biotechnology and Biosciences (University of Milano-Bicocca, P.zza della Scienza 2, Milano, Italy).*
[2] *Dept of Medicine and Surgery (University of Milano-Bicocca, Via Cadore, 48, Monza, Italy)*

e-mail: cristina.airoldi@unimib.it

Bombesin (BN) is a 14-residue peptide originally isolated from the amphibian Bombina bombina[1]. It belongs to a family of peptides showing a variety of biological activities in numerous tissues and cell types,[2] exerted through their interaction with the Gastrin-Releasing Peptide Receptors (GRPR), transmembrane G-proteins coupled receptors triggering different signaling transduction pathways, resulting, among which, in the stimulation of cell proliferation. GRPRs are significantly involved in the pathogenesis of different human cancers[3], and are recently emerged as tumoral markers in early prostate and breast cancers diagnosis[4]. For these reasons, the research of new GRPR ligands as antagonists or carriers for cytotoxic and imaging molecular tools might be a promising strategy for the treatment and diagnosis of human tumoral malignancies[5]. In this scenario, structural data about BN binding to GRPR are required for the design and synthesis of high affinity receptor ligands, but, unfortunately, they are not yet available. BN conformation has been studied in various solvents demonstrating that it adopts an unordered structure in aqueous media and in dimethyl sulfoxide[6], while a partial helical structure has been observed in aqueous solutions containing TFE[7]. According to proposed models, this is the conformation that, probably, BN presents when anchored to biological membranes.

With the aim to verify this hypothesis, we studied the effect of d_{25}-SDS (a biological membrane mimetic) on BN by CD and NMR spectroscopy. As for BN-GPCR interaction, the heptapeptide BN(8–14) has been shown to be the minimal carboxyl fragment interacting with the receptor, the same experiment were performed also on the BN C-terminal heptapeptide. Moreover, to discover the structural determinants of BN interaction with GRPR, the binding of both BN and BN(8-14) to human prostate carcinoma cell line (PC-3) over-expressing the receptor has been studied thought on-cell STD-NMR experiments.

In addition, we synthesized a library of ligands based on a rigid and spatially defined selected glycidic scaffold, differing for the nature of the potential pharmacophoric moieties. The biological activity of these compounds was preliminary screened by evaluating their ability to modulate the level of cytosolic Ca^{2+} (agonist or antagonist activity) in PC-3 cell.

Authors acknowledge AIRC for funding project 17030 - Targeting of Gastrin-Releasing Peptide receptor expressing tumors: NMR characterization of Bombesin/GRP-R interaction.

1. Anastasi, A.; Erspamer, V.; Bucci, M., *Experientia* **1971,** *27* (2), 166-167.
2. Bruzzone, R., *European Journal of Biochemistry* **1989,** *179* (2), 323-331.
3. Ramos-Álvarez, I.; Moreno, P.; Mantey, S. A.; Nakamura, T.; Nuche-Berenguer, B.; Moody, T. W.; Coy, D. H.; Jensen, R. T., *Peptides* **2015,** *72*, 128-144.
4. Mansi, R.; Fleischmann, A.; Macke, H. R.; Reubi, J. C., *Nat Rev Urol* **2013,** *10* (4), 235-244.
5. Hohla, F.; Schally, A. V., *Cell Cycle* **2010,** *9* (9), 1738-1741.
6. Carver, J. A., *European Journal of Biochemistry* **1987,** *168* (1), 193-199.
7. (a) Carver, J. A.; Collins, J. G., *European Journal of Biochemistry* **1990,** *187* (3), 645-650; (b) Díaz, M. D.; Fioroni, M.; Burger, K.; Berger, S., *Chemistry-A European Journal* **2002,** *8* (7), 1663-1669.

Urease inhibition by gold compounds

Marta Palombo[1], Angela Casini[2] and Stefano Ciurli[1].

[1] *Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy.*
[2] *School of Chemistry, Cardiff University, Park Place, Cardiff, UK*.

e-mail: marta.palombo2@unibo.it

Urease is a nickel-dependent enzyme that catalyses the hydrolysis of urea to yield ammonia and carbamate. The hydrolysis of the reaction products induces an overall pH increase, that makes urease a virulence factor of several human pathogens, in addition decreases the efficiency of soil organic nitrogen fertilization[1]. Therefore, efficient urease inhibitors are actively sought. In this work is reported a kinetic characterization of the interaction between urease from *Canavalia ensiformis* (jack bean, JBU) with different gold compounds. The medicinal chemistry of gold compounds is based on the high affinity of gold for protein thiols and selenols[2], this together with the peculiarity of the urease to have a key cysteine, located on the mobile flap and regulating the substrate access to the active site[3], makes gold(III) complexes potentials targets for new urease inhibitors, with possible positives repercussions in human health.

1. Zambelli, B., Musiani, F., Benini, S. and Ciurli, S. *Accounts of chemical research* **2011**, *44*, 520-530;
2. Berners-Pricea, S.J. and Filipovska, A. *Metallomics* **2011**, *3*, 863–873;
3. Mazzei, L., Cianci, M., Musiani, F. and Ciurli, S., *Dalton Trans*., **2016**, *45*, 5455.

A personal journey in the application of biocatalysis to the synthesis of "fine chemicals"

Oreste Piccolo

Studio di Consulenza Scientifica

Via Bornò 5, 23896 Sirtori (LC)

e-mail: contact@scsop.it

www.scsop.it

It is a personal journey, but obviously always working together with many valuable colleagues and collaborators, that begins in the '90s with an industrial request of a company, of which I was consultant, to develop the synthesis of some phospholipids using not commercially available phospholipase D,[1] but which then has continued in applied researches to the preparation of various *fine chemicals* at the university of Venice and Pisa, often motivated by my personal curiosity or by the desire to identify potentially greener and more sustainable synthetic protocols. The journey unfolded through the asymmetric reduction with enoate reductases and alcohol dehydrogenases for the production of some enantioenriched fragrances,[2] up to biocatalytic kinetic resolution of some esterified alcohols with immobilized lipases[3] and asymmetric synthesis of some optically pure amines with immobilized transaminases.[4] Some relevant intermediates of pharmaceutical interest were obtained, as well as a potential bifunctionalized monomer for bio-plastics.[5] Even more recently the use of plant tissues, as alternative reduction biocatalysts, has stimulated my interest.[5] During these years I was often engaged with the difficulty of developing a process, even at the level of feasibility study, due to the problem of buying some enzymes and/or of their cost, I had to evaluate the advantage / disadvantage of using free or immobilized enzymes and the need for the latter to recycle them, and, in the case of plants, to overcome the problems of productivity, seasonal variability and supply. Some of the more significant examples will be presented with data often unpublished or disclosed only in conferences, in order to illustrate some skills and stimulate / start, if possible, new collaborations.

[1] De Ferra, L.; Massardo, P.; Piccolo, O.; Servi, S. *US5700668*(B)
[2] Piccolo, O.; Cardinali, L.; Sartorato, E.; Paganelli, S. *WCCAS-2010*, Beijing (2010)
[3] Tofani, G.; Petri, A.; Piccolo, O.; *Tetrahedron Asymmetry*, **26** , 638–643 (2015)
[4] Piccolo, O.; Colonna, V.; Petri, A. *6thEuCheMS Chemistry Congress*, Seville (2016)
[5] Piccolo, O.; Petri, A.; Masia, G. *3rd EuCheMS Congress on Green and Sustainable Chemistry*, York (2017); work submitted for publication (2018)

An insight into the structural dynamics of an essential Urease chaperone: UreG from *H. pylori*.

Annalisa Pierro, [1,2] Bruno Guigliarelli, [2] Valérie Belle,[2] Elisabetta Mileo[2] and Barbara Zambelli[1]

[1]*Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology (University of Bologna).*
[2]*Aix Marseille Univ, CNRS, BIP, Bioénergétique et Ingénierie des Protéines, IMM, Marseille, France.*

e-mail: annalisa.pierro2@studio.unibo.it

UreG is a P-loop GTP hydrolase involved in the maturation of the nickel-containing urease, an essential enzyme found in plants, fungi, bacteria and archaea. This protein couples the hydrolysis of GTP to the delivery of nickel into the active site of apo-urease, interacting with other urease chaperones in a multi-protein complex necessary for enzyme activation. Two "conformational states" of UreG can be found: a well-structured conformation was reported by crystallography [1;2], while in solution the protein adopts a disordered and flexible form, making it the first documented case of intrinsically disordered enzyme[3]. What is the active form of the enzyme? Does it change *in vivo*?

We addressed these questions using Site-Directed Spin Labelling (SDSL) coupled to Electron Paramagnetic Resonance (EPR), a technique that allowed us to dissect the conformational landscape of UreG from *Helicobacter pylori*. SDSL-EPR is based on the introduction of a paramagnetic label (usually a nitroxide radical) at a selected site of a protein followed by it analysis by EPR spectroscopy[4]. The dynamics of the spin label is related to the structural properties of the protein under investigation and can be used to follow protein's structural changes[5]. Furthermore, of particular interest are inter-label distance measurements at long-range (18-80 Å) that can be performed using two spin labels. Such measurements rely on the dipole-dipole coupling between spin labels that can be measured by pulsed double electron-electron resonance (DEER) techniques.

Thanks to a careful site-directed mutagenesis and labeling work, we were able to investigate by EPR spectroscopy several regions of UreG in the absence and in the presence of known ligands (GTP, GDP, nickel, bicarbonate). In particular, UreG structural changes obtained by EPR clearly showed which region is more affected by ligand binding.

With the aim of studying the structural dynamics of UreG in conditions close to physiological/cellular ones, preliminary outcomes of SDSL-EPR *in cell* will be presented.

1. Fong Y.H. *et al.*; "Structure of UreG/UreF/UreH Complex Reveals How Urease Accessory Proteins Facilitate Maturation of Helicobacter pylori Urease", *PLoS Biol* **2013** *11*(10):e1001678. DOI:10.1371/journal.pbio.1001678
2. Hon, M *et al.*; "Structural insights into how GTP-dependent conformational changes in a metallochaperone UreG facilitate urease maturation", *PNAS* **2017**, doi:10.1073/pnas.1712658114.
3. Zambelli B *et al.*; "Zn^{2+} -Linked Dimerization of UreG from *Helicobacter Pylori* , a Chaperone Involved in Nickel Trafficking and Urease Activation", *Proteins* **2008**: 222–39. DOI:10.1002/prot.
4. Le Breton *et al;* "Exploring Intrinsically Disordered Proteins Using Site-Directed Spin Labeling Electron Paramagnetic Resonance Spectroscopy." *Frontiers in Molecular Biosciences* 2 (2015): 21. DOI:10.3389/fmolb.2015.00021.
5. M. Palombo *et al.*; "The relationship between folding and activity in UreG, an intrinsically disordered enzyme." *Sci. Rep.* **2017**, *7*, 5977. DOI:10.1038/s41598-017-06330-9

Ligninolytic enzymes: insights on the catalytic mechanism, enzymatic immobilization and LCA analysis

Rebecca Pogni

Department of Biotechnology, Chemistry and Pharmacy - Università di Siena, Via A. Moro 2 - Siena (Italy)

e-mail: rebecca.pogni@unisi.it

Lignin removal is a key step for carbon recycling in terrestrial ecosystems, as well as a central issue for industrial utilization of plant biomass[1]. Lignin is the second most abundant polymer in nature. In nature, white-rot fungi are the main organisms responsible for lignin biodegradation, a process that has been described as an enzymatic combustion. These fungi have developed extracellular ligninolytic machinery made up of oxidoreductases including peroxidases, laccases and oxidases, among others. The catalytic mechanism of these oxidative enzymes led to the break of lignin bonds or to the formation of new ones by radical condensation, or even indirectly to oxygenation reactions. All these reactions are of interest from a biotechnological point of view[2-4].

The interest in biocatalysts for biotechnological applications continues to grow. Nonetheless the fragile nature, high cost, and high loadings of enzymes required for commercial production limits the use of free enzymes. Enzyme immobilization is utilized to surmount the stability, recovery, and recyclability disadvantages of using enzymes in solution, making them industrially and commercially viable[4,5]. Nanomaterials are particularly suitable for enzymatic immobilization. Among nanomaterials, nanofibers represent one of the most attractive nano-device for the production of high added value products. When the diameters of polymer fiber materials are shrunk from micrometers to sub-microns or nanometers there appear several amazing characteristics such as very large surface area to volume ratio, flexibility in surface functionalities, and superior mechanical performance (stiffness and tensile strength) compared with any other known form of the material. Membranes of electrospun nylon and polyurethane nanofibers, mesoporous silicas, or magnetic nanoparticles can be synthesized, functionalized and used for enzymatic immobilization.

Fungal laccase in its immobilized form has been used for the synthesis of novel dyes for textile applications in the frame of the Eco-innovation european project BISCOL (ECO/09/256112) proposing a novel dyeing process as an alternative for a more eco-efficient production of coloured textiles[6]. Bio-synthetic pathway for dyestuff production represents a high-tech approach that exploits the ability and efficiency of enzymes to catalyse the formation of new dyes. A comparative LCA analysis to assess and compare the environmental impacts of two dyeing processes was performed.

1. Ragauskas, A.J.; et al. *Science* **2014**, *311*, 484-489.
2. Pogni, R.; Baratto, M.C.; Sinicropi, A.; Basosi R. *Cell. Mol. Life Sci.* **2015**, *72*, 885-896.
3. Saez-Jimenez, V.; Baratto, M.C.; Pogni, R.; Rencoret, J.; Gutiérrez, A.; Santos, J.I.; Martínez, A.T.; Ruiz Dueñas, F.J. *J. Biol. Chem.* **2015**, *290*, 23201-23213
4. Harir, M.; Bellahcene, M.; Baratto, M.C.; Pollini, S.; Rossolini, G.M.; Trabalzini, L.; Fatarella, E.; Pogni, R. *J. Biotechnol.* **2018**, *265*, 54-64
5. Fatarella, E.; Spinelli, D.; Ruzzante, M.; Pogni, R. *J. Mol. Catal. B: Enzymatic* **2014**, *102*, 41.
6. Parisi, M.R.; Fatarella, E.; Spinelli, D.; Pogni, R.; Basosi, R. *J. of Cleaner Production* **2015**, *108*, 514-524

Self-assembling nucleic acid analogues for the fabrication of new fluorescent materials

Alessandra Romanelli[1], Concetta Avitabile[2], Antonella Accardo[3]

[1]*Dipartimento di ScienzeFarmaceutiche-Università di Milano- via Venezian 21-20133 Milan*
[2] *Istituto di Biostrutture e Bioimmagini, CNR, via Mezzoacannone 16-Napoli*

[3]*Dipartimento di Farmacia Università di Napoli "Federico II", via Mezzocannone 16, 80134 Napoli.*

e-mail: alessandra.romanelli@unimi.it

Self-assembling of molecules by non-covalent interactions is one of the most attracting topics in supramolecular chemistry. The use of short peptides or modified nucleotides as building blocks of the aggregates is particularly intriguing, as these are very easy to synthesize; moreover subtle changes in the chemical structure of such building blocks may drastically affect the properties of the aggregates. The self-assembly of nucleobases has been extensively investigated with the aim to produce new materials, electronic nanodevices and biosensors.[1] The ability of Peptide Nucleic Acids to aggregate is yet very little explored, despite its practical applications. Recently Gazit described the ability of PNA dimers to self-assemble into organized structures, guided by stacking interactions and Watson-Crick base pairing.[2]. GC dimers exhibit interesting fluorescent properties, being able to strongly emit in the wavelength range between 420 and 490 nm, upon excitation between 330 and 430 nm. In our lab we're actually exploring the ability of short PNA sequences, PNA-peptide conjugates to form aggregates. Fluorescence properties of these molecules are under investigation.

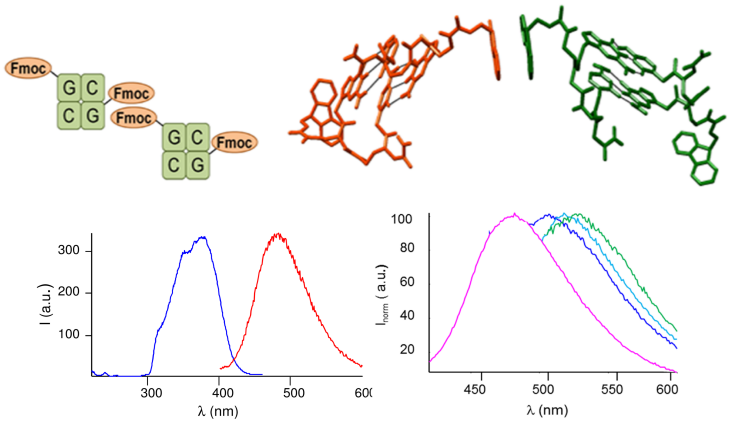


Figure 1: top: scheme of the structure of the Fmoc-GC aggregates; bottom: fluorescence spectra of Fmoc-GC in organic solvents

1. J. T. Davis and G. P. Spada, Chemical Society reviews 2007, 36, 296-313. XXXX, X.; YYYYYY, Y. J. Med. Chem. 2002, 12, 6500-6521.
2. 2. O. Berger, L. Adler-Abramovich, M. Levy-Sakin, A. Grunwald, Y. Liebes-Peer, M. Bachar, L. Buzhansky, E. Mossou, V. T. Forsyth, T. Schwartz, Y. Ebenstein, F. Frolow, L. J. W. Shimon, F. Patolsky and E. Gazit, Nature Nanotechnology 2015, 10, 353-360.

Polyhydroxyalkanoates: production, extraction and novel applications

Chiara Samorì[1], Paola Galletti,[1] Cristian Torri,[1] Emilio Tagliavini[1]

[1]*Dipartimento di Chimica "Giacomo Ciamician", via Selmi 2, Bologna*

e-mail: chiara.samori3@unibo.it

Polyhydroxyalkanoates (PHAs) are promising polyesters, produced by bacteria through aerobic fermentation of various carbon sources. These biopolymers are completely biodegradable under aerobic and anaerobic conditions and have elastomeric/thermoplastic properties, which are tunable according to actual co-monomer composition. However, despite the efforts put towards the development of cost-effective fermentative systems, PHA production cost still remains considerably high (~5-6 $/kg), hampering the exploitation of these biopolymers as commodity materials. In recent years, it has been claimed that the use of mixed microbial cultures (MMCs) instead of single strains (e.g. *Cupriavidus necator*) could represent a cheaper strategy for producing PHAs. Moreover, the availability of new methodologies for achieving a sustainable and economic recovery of PHA from microbial biomass is a key aspect; in fact, the downstream cost can cover almost 50% of the total production costs, involving an extensive use of non-recyclable (and sometimes highly toxic) chemicals/ materials and a high energy consumption.

Herein, we propose a novel route for sustainably achieving PHAs by combining the use of: i) fermented waste biomass as carbon source for PHA-producing bacteria, ii) a fully automated bioreactor for cultivating these bacteria, and iii) green protocols for the extraction of PHA from single strains bacteria and MMCs. Specifically, these protocols are mainly based on the use of green organic solvents such as dimethyl carbonate (DMC) and the bio-based γ-valerolactone (GVL), and green Switchable Anionic Surfactants (SAS), soluble in H_2O at alkaline pH (condition at which they are anionic), but insoluble, and thus easily recoverable, at lower pH. The addition and removal of CO_2 to the system represents a simple and effective way to achieve the pH switch.[1,2]

Additionally, the possibility of obtaining PHA-gels by using bio-based green solvents such as GVL, ethyl lactate or biodiesel, is exploited for creating a novel eco- and user-friendly tool, useful for cleaning painting surfaces and bronze from aged patinas and protective resins, therefore providing a sustainable approach to conservation of Cultural Heritage, beneficial not only for the artworks, but also for human health and the environment.[3,4]

1. Samorì, C.; Basaglia, M; Casella, S.; Favaro, L.; Galletti, P.; Giorgini, L.; Mazzocchetti, L.; Torri, C.; Tagliavini, E. *Green Chem*. **2015**, *17*, 1047.
2. Samorì, C.; Abbondanzi, F.; Galletti, P.; Giorgini, L.; Mazzocchetti, L.; Torri, C.; Tagliavini, E. *Bioresource Technol*. **2015**, *189*, 195.
3. Samorì, C.; Galletti, P.; Giorgini, L.; Mazzeo, R.; Mazzocchetti, L.; Prati, S.; Sciutto, G.; Volpi, F.; Tagliavini, E. *ChemistrySelect* **2016**, *1*(15), 4502.
4. Prati, S.; Volpi, F.; Fontana, R.; Galletti, P.; Giorgini, L.; Mazzeo, R.; Mazzocchetti, L.; Samorì, C.; Sciutto, G.; Tagliavini, E. *Pure Appl. Chem*. **2017** doi.org/10.1515/pac-2017-0507

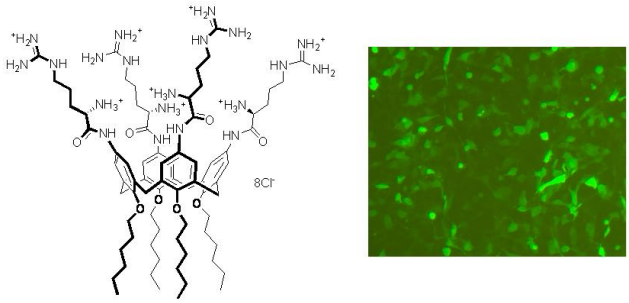
Ammonium containing calixarenes as multivalent systems for the delivery of nucleic acids and mimics

Francesco Sansone[1], Alessia Finotti[2], Michela Lomazzi[1], Jessica Gambarello[2], Alex Manicardi[1], Alessandro Casnati[1], Roberto Corradini[1], Roberto Gambari[2]

[1]*Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parco Area delle Scienze 17/a, 43124 Parma, Italy*.
[2]*Dipartimento di Scienze della vita e biotecnologie, Università di Ferrara, Via Fossato di Mortara 74, 44121 Ferrara, Italy*.

e-mail: francesco.sansone@unipr.it

Gene therapy is based on the possibility of delivering proper nucleic acids or mimics into the cells in order to block or restore processes and activities related with alterations in the genome of the patients. The first recent successes in this field exploit suitably modified viruses as carriers. However, their use is related with some possible drawbacks such as inflammation, toxicity, mutagenesis, limits in the size of the cargos, expensive procedures for preparation in large scale. For this reasons it is still currently very active and intense the research aimed at developing non viral vectors based on organic molecules and polymers working as safe and efficient delivery systems. In this context, since some years we are designing and synthesizing molecular vectors characterized by a multivalent exposition of active units linked to a calixarene scaffold.[1] The active units are mainly constituted by ammonium groups, combined with lipophilic chains that confer to the vector a well balanced amphiphilic character necessary for the transfection activity. Some of these calixarene based vectors show a very high efficiency in the transfection activity associated with a very low or negligible citotoxicity, resulting better than commercially available formulations for transfection protocols. Together with an impressive ability in the delivery of DNA, more recently we could verify and demonstrate a remarkable activity of these macrocyclic vectors in the transfection of cells also with RNA, such as miRNA and pre-miRNA,[2] and nucleic acid mimics such as Peptide Nucleic Acids (PNAs). It is more than notewhorty that for the latter ones no other significant vectors are currently available despite the relevance of these molecules as potential therapeutics.[3] These new findings make our multivalent vectors built on a calixarene scaffold very useful and promising non viral transfecting agents of interest for researchers and companies working on gene therapy and development of drugs based on nucleic acids and mimics.



A fluorescence microscopy image of cells (right) transfected with the EGFP plasmid (encoding for the green fluorescence protein) by using a calixarene based vector (left)

1. Bagnacani, V.; Franceschi, V.; Bassi, M.; Lomazzi, M.; Donofrio, G.; Sansone, F.; Casnati, A.; Ungaro, R. *Nat. Commun.* **2013**, 4:1721. DOI: 10.1038/ncomms2721.
2. Patent pending
3. Patent pending

MULTIVALENT CALIXARENE-BASED MOLECULES FOR THE INHIBITION OF CARBONIC ANHYDRASES ENZYMATIC ACTIVITY

Davide Sbravati[1], Valeria Pizzolante[1], Silvia Bua[2], Fabrizio Carta[2], Alessandro Casnati[1], Francesco Sansone[1], Claudiu T. Supuran[2]

[1] *Department of Chemistry, Life Sciences and Environmental Sustainability, Università di Parma (Parco Area delle Scienze 17/a, 43124, Parma, Italy);*

[2] *Neurofarba Department, Università di Firenze (Via Ugo Schiff 6, Polo Scientifico, 50019 - Sesto Fiorentino-Firenze, Italy);*

e-mail: davide.sbravati@studenti.unipr.it

Calixarenes are synthetic macrocyclic, oligo-phenolic compounds very popular in supramolecular chemistry as receptors. Recently they started to be widely employed in bio-macromolecule targeting such as nucleic acids, enzymes, proteins.[1] Differently to traditional drugs, calixarenes can be functionalized in several ways exposing active units, for instance pharmacophores, in multiple copies and different orientations in space. This feature allows to exploit the so called "multivalency effect" that can result in a complexation activity towards the biological target significantly more efficient with respect to analogous monomeric ligands. Usually, multivalent ligands as those based on calixarene scaffolds show the beneficial effects of multivalency in the binding to macromolecules presenting multiple copies of equivalent recognition sites.[2] However, recently some attempts have been done to verify this effect in the inhibition of enzymes.[3] In this context, we have prepared a preliminary small library of potential inhibitors for Carbonic Anhydrases (CAs) based on calixarenes and exposing multiple copies of benzenesulfonamide (*e.g.* ***Figure* 1**). These compounds have been tested towards six different CA isoforms[4] (hCAI, hCAII, hCAIX, VchCAβ, Can2, MgCA) and compared with two monomeric analogues and acetazolamide. Some derivatives have shown K_i values in the μM-nM range. Synthesis of the ligands and inhibition studies will be reported in this presentation.

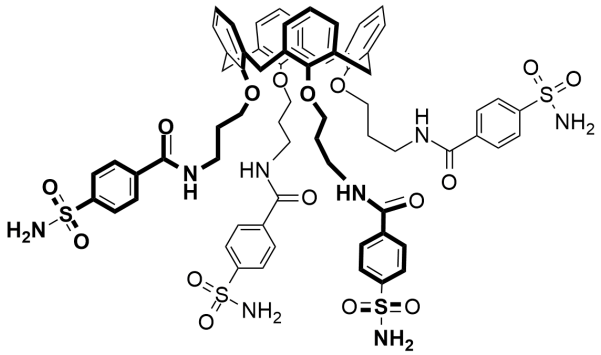


Figure 1. Example of benzosulfonamidecalix[4]arene derivative.

1. GIULIANI, M.; MORBIOLI, I.; SANSONE, F.; CASNATI, A. Chem. Commun. **2015**, *51*, 14140-14159.

2. SANSONE, F.; CASNATI, A. Chem. Soc. Rev. **2013**, *42*, 4623-4639.

3. a) RÍSQUEZ, R.C.; FERNANDEZ, M.J.G.; NIERENGARTEN, J.F.; MELLET, C.O. Chem. Eur. J., **2013**, 19, 16791– 16803; b) ABELLÁN-FLOS, M.; TANÇ, M.; SUPURAN, C.T.; VINCENT P.; S. Org. Biomol. Chem., **2015**, *13*, 7445-7451.

4. a) ALTERIO, V.; DI FIORE, A.; D'AMBROSIO, K.; SUPURAN, C.T.; DE SIMONE, G. Chem. Rev., **2012**, *112*, 4421-4468; b) SUPURAN, C.T.; SCOZZAFAVA, A.; JURCA, C.B.; ILIES, A.M. Eur. J. Med. Chem., **1998**, 83-93.

Playing chemistry with food proteins: molecular tools for food safety, food authenticity and food waste valorization

Tullia Tedeschi[1], Barbara Prandi, Sofie Buhler, Cecilia Anzani, Arnaldo Dossena and Stefano Sforza

[1]*University of Parma, Food and Drug Department*

e-mail: tullia.tedeschi@unipr.it

Molecular characterization of proteins, at the different level (sequence, structure, modifications) can be a very important task in bioorganic and food chemistry. Moreover, the possibility of chemically modifying protein extracts (such as in the enzymatic production of hydrolizates or in the chemical addition of new functional groups) can be used in many research areas, such as food safety (food allergens identification), food authentication (generation of species-specific petpides) and recently for the valorization of food wastes (isolation of protein-rich biomasses with high quality proteins of high nutritional value).

Concerning food allergen identification proteomic tools, such enzymatic digestion of pure proteins or mixtures followed by mass spectrometry analysis, we achieved the complete molecular characterization of LTP, which is an important food allergen, in different vegetal sources (peach, apricot, almond). Similar studies were also applied for the identification of gluten in different wheat products. In both case a detailed study of the stability of the allergen to the gastro-intestinal digestion was also tested by applying simulated digestion models and analyses of the digestates by LC-MS. Chemical peptide synthesis has been also approached in order to have standards for molecular markers quantification. Finally, chemical ligation strategies were also explored for the first total synthesis of a food allergen. Synthetic protein fragments were also used for assessing allergenic tests.

The identification of the origin of peptide mixtures is also very important in order to assess the origin of a food product. With this aim we performed the species authentication in an high processed food, i.e. Bolognese sauce. By using two different marker peptides, specific for beef and pork meat, both deriving from α2-collagen chain, and their detection by LC/MS, the two species were detected and accurately identified in several sauce samples.

Recently, the production of protein hydrolysates from recalcitrant industrial meat waste (fleshing) has been investigated in order to obtain amino acids and peptides mixtures to be valorised as potential additives in feed and food industry. The activity of six proteolytic enzymes was tested in order to determine their efficiency in solubilizing fleshing. The composition of the nitrogen fraction of the hydrolysates was characterized at the molecular level. The results showed that papain and alcalase are the most efficient enzymes, totally degrading fleshing by generating soluble hydrolyzates rich in peptides and amino acids.

All these results indicate that the ability to isolate, characterize, modify and de novo sytnehsize food proteins and peptides can have important applications in food industry.

Chemical modification and structural characterization of Neuroserpin

Mattia Vacchini,[1] Carlo Santambrogio,[1] Rosaria Russo,[2] Rita Grandori,[1] Stefano Ricagno,[2] Laura Cipolla[1]

[1] Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano-Italy;
[2] Dept. of Bioscience, University of Milano, Via Celoria 26, 20133 Milano-Italy
e-mail: m.vacchini@campus.unimib.it

Neuroserpin (NS) is a serine-protease inhibitor involved in neurodegeneration.[1,2] The aim of this work is to study the covalent labeling of NS in its monomeric and polymeric forms, in an attempt to map protein surface areas which may be involved in the polymerization process standing at the base of neurological diseases associated with NS (i.e., Alzheimer's disease, Familial Encephalopathy with Neuroserpin Inclusion Bodies, FENIB). Preliminary works have been done: NS has been lysine-methylated via reductive amination with formaldehyde and sodium cyanoborohydride in potassium phosphate buffer, in an explorative reaction thought to test the feasibility of the methodology, looking for chemical conditions compatible with NS folding. NS lysine-methylation has been assessed via mass spectrometry and methylated NS folding and polymerization propensity have been investigated by size exclusion chromatography and circular dichroism. Mass spectrometry analyses on intact and proteolytically digested monomeric NS, indicate an almost complete dimethylation of lysine residues after 20 seconds of reaction. Structural analyses of native and methylated NS showed no major conformational changes or aggregation effects after chemical modification. Further developments in this research will include optimizing purification protocols/reaction conditions and mapping NS interaction surfaces involved in polymerization by comparing the degree and distribution of covalent labeling between NS monomeric and polymeric forms.

[1] *Semin Cell Dev Biol.* ***2017*** *Feb;62:152-159. doi: 10.1016/j.semcdb.2016.09.007.*

[2] *Learn Mem.* ***2017*** *Nov 15;24(12):650-659. doi: 10.1101/lm.045864.117.*

In-cell targeting of activity and maturation of *Helicobacter pylori* urease as a novel strategy for antibacterial drug screening

Barbara Zambelli, Cinzia Tarsia, Francesca Florini, Paolo Cinelli, Alberto Danielli, Stefano Ciurli

Department of Pharmacy and Biotechnology, University of Bologna

e-mail: barbara.zambelli@unibo.it

Helicobacter pylori is a Gram-negative bacterium that colonizes the stomach of half of the human population and is strongly associated with peptic ulcer and gastric malignancies, such as stomach cancer. Accordingly, in 1994 *H. pylori* was the first, and so far, unique bacterium classified as a class I carcinogen by IARC, and in 2013 its eradication was recommended by WHO as a strategy to diminish the incidence and effects of gastric-associated tumors[1].

Living in the strongly acidic environment of the gastric niche, *H. pylori* developed unique adaptive mechanisms to neutralize extremely low pH for surviving in such challenging setting. To this aim, the most important player is the nickel-dependent urease, whose catalytic activity produces an increase of the pH of the micro-environment around the bacterium[2,3]. Urease is not present in the human proteome, thus representing a promising target to develop drugs against *H. pylori*.

The present work aims to provide an innovative strategy for the identification of new antibacterial molecules that can open the route to eradication of *H. pylori* by targeting the urease activity. An in-cell urease assay was developed in the organism model *E. coli* to test specific enzyme inhibitors that selectively bind the active site of urease, or that interrupt the protein-protein interaction (PPI) network that deliver nickel into the active site, thus preventing enzyme maturation. For the latter, a two plasmids system was produced for in-cell screening of peptide-based inhibitors. This system is expected to provide the identification of promising candidates that constitute the basis for developing new and innovative drugs, to be used, after further experimental evaluation, in *H. pylori* eradication programs.

1. IARC W. G. Reports, *Helicobacter pylori* eradication as a strategy for Preventing Gastric Cancer. **2013**. Volume 8.
2. Zambelli, B.; Musiani F.; Benini; S. Ciurli, S. *Acc. Chem. Res.* **2011**, 44(7): 520-530.
3. Zambelli, B.; Ciurli S. (2013). *Met. Ions Life Sci.* **2013,** 13: 321-357.