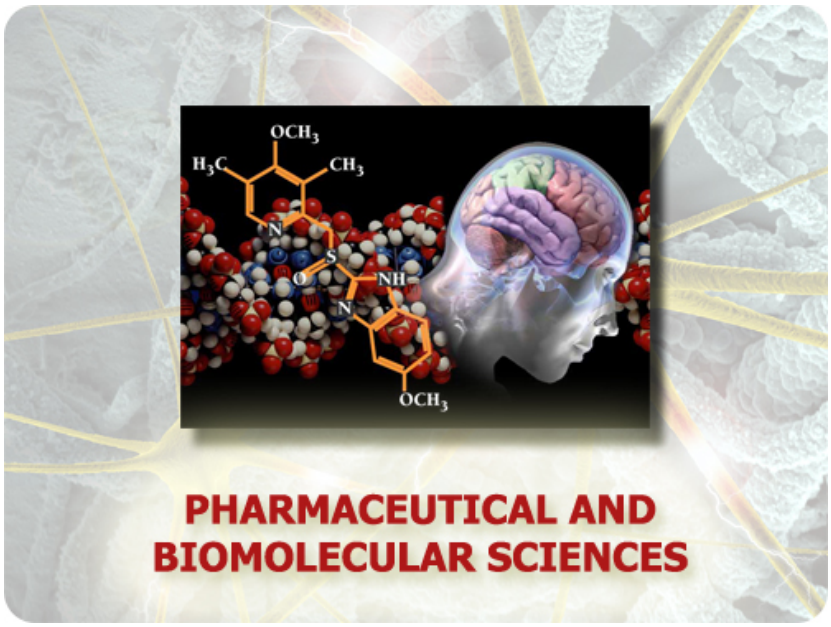
Università degli Studi di Torino

Scuola di Dottorato in Scienze della Natura e Tecnologie Innovative

Dottorato in Scienze Farmaceutiche e Biomolecolari

Advances in



at the University of Torino

Presentazione dei risultati dei progetti di ricerca
dei Dottorandi e delle Dottorande del XXIX ciclo

Aula Magna del Rettorato, via Po 17

Venerdì 18 novembre 2016

Le Dottorande ed i Dottorandi del 29° ciclo del Dottorato in Scienze Farmaceutiche e Biomolecolari si presentano al territorio

Da sempre il Dottorato in Scienze Farmaceutiche e Biomolecolari organizza delle giornate residenziali dedicate a ciascuno dei cicli attivi, al termine di ogni anno di attività, come momento di verifica del lavoro svolto nell'ambito di ciascun progetto, e come occasione di incontro e confronto di tutta la comunità scientifica del Dottorato.

Un'inattesa variazione della sede dell'incontro precedente a questo, che ci ha visti ospiti dell'Unione Industriale (che ringraziamo nuovamente), è stata l'occasione per immaginare una organizzazione diversa della giornata dedicata ai Dottorandi ed alle Dottorande del 29° ciclo, che concludono a dicembre 2016 i loro progetti di ricerca triennali. In particolare, si è voluto invitare a questa giornata le aziende ed i Poli di Innovazione che operano nella nostra Regione in settori in cui trovano applicazione le ricerche presentate (e qui rinnoviamo i ringraziamenti all'Unione Industriale, e li estendiamo allo Sportello Università-Impresa del nostro Ateneo), in modo che possano apprezzare il valore di questi giovani ricercatori.

L'invito è stato doverosamente esteso alle aziende ed alle fondazioni bancarie, Compagnia di San Paolo e Fondazione CRT, che tramite convenzioni, contratti di apprendistato, borse di studio hanno sostenuto un numero significativo dei progetti presentati oggi: li ringraziamo per la fiducia riposta, ed in questa occasione potranno avere un riscontro dei frutti dei loro investimenti.

Ringraziamo anche il Magnifico Rettore, che ha voluto mettere a disposizione per lo svolgimento di questa giornata l'Aula Magna del Rettorato, che oggi vede protagonisti giovani che hanno scelto l'Università di Torino per la loro formazione alla ricerca scientifica.

Sperando che questa giornata sia la prima edizione di un appuntamento annuale di contatto e conoscenza reciproca tra attori di primo piano del territorio per la promozione e l'attuazione dell'innovazione ed il Dottorato in Scienze Farmaceutiche e Biomolecolari, abbiamo pensato di inserire in questo fascicolo altri elementi di presentazione del Dottorato stesso. Sono quindi elencati i progetti di ricerca delle Dottorande e dei Dottorandi degli cicli attivati successivamente al 29° (ad oggi siamo giunti al 32°), e sono state riportate sintetiche schede informative sulle strutture dove questi giovani svolgono il loro percorso di formazione alla ricerca, e cioè i cinque Dipartimenti (Biotecnologie e Scienze per la Salute, Chimica, Scienza e Tecnologia del Farmaco, Scienze della Sanità Pubblica e Pediatriche, Scienze della Vita e Biologia dei Sistemi) che conferiscono a questo Dottorato un peculiare carattere multidisciplinare.

Concludo, porgendo, a nome di tutta la comunità del nostro Dottorato, un sincero benvenuto a quanti hanno potuto rispondere all'invito a partecipare alla giornata, che ci auguriamo ricca di spunti e occasioni di interazione.

Il coordinatore del Dottorato
in Scienze Farmaceutiche e Biomolecolari

Prof. Gianmario Martra

SOMMARIO

PROGRAMMA

I riassunti in formato elettronico sono disponibili in un'apposita sezione della home page del sito web del Dottorato:

http://dott-sfb.campusnet.unito.it

ORARIO	DOTTORANDA/O	PRESENTAZIONE	SETTORE
9.00-9.15		***Apertura dei lavori***	
9.15- 9.45	Arizzi Maria Concetta	Characterisation of novel hydrogenases from waste biomasses for application as H_2 producing catalysts	Biomasses, Energy
9.45-10.15	Caporaso Marina Nunzia	High energy efficiency chemical processes for eco-friendly synthetic applications	Green Chemistry
10.15-10.45	Cirrincione Simona	Investigation on metabolites produced by Lactic Acid Bacteria of food interest	Food
10.45-11.00		***coffee break***	
11.00-11.30	Fornasero Stefania	Epidemiological and molecular study of pathogenicity characters of enterobacteriaceae isolated from humans and foods	Public health, Food
11.30-12.00	Magagna Federico	Advanced analytical approaches for "sensomic" investigations of high quality food matrices of vegetable origin	Food
12.00-12.30	Ruzza Marta	Innovative NMR bio-analytical application	Bio-analytics
12.30-13.00	Capozza Martina	Nanosized systems for imaging, a focus on photoacoustic imaging	Health (diagnostics, surgery)
13.00-14.00		***Pranzo***	
14.00-14.30	Cavaletto Noemi	Functional characterization of the US12 gene family of the Human Cytomegalovirus	Health (pharma, biotech)
14.30-15.00	Cocco Mattia	Design, synthesis and pharmacological characterization of new chemical entities targeting NLRP3 inflammasome activation and related signaling pathways	Health (pharma, biotech)
15.00-15.30	Consolino Lorena	Multi-parametric MRI studies in murine tumour models	Health (diagnostics)
15.30-16.00	Ferrante Terenzio	Role of 4-methyl sterols in post-squalene metabolic diseases and cancers, Hedgeogh protein pathway depending	Health (pharma)
16.00-16.15		***coffee break***	
16.15-16.45	Marengo Alessandro	Liposomes for the active targeting of anticancer drugs against receptors overexpressed on cancer stem cells for the treatment of pancreatic adenocarcinoma	Health (pharma)
16.45-17.15	Ruggiero Maria Rosaria	New nanosized agents for imaging guided treatment of tumours	Health (personalized medicine, biotech)
17.15-17.45	Sainas Stefano	Targeting the human Dihydroorotate Dehydrogenase (hDHODH) using hydroxylated azole scaffolds inside a Scaffold Hopping Bioisosteric approach	Health (pharma), agro/chemistry
17.45-18.00		***Chiusura dei lavori***	

Abstract
dei progetti di ricerca
delle Dottorande e dei Dottorandi
del 29° ciclo

(2013-2016)

Characterisation of novel hydrogenases from waste biomasses for applications as H_2 producing catalysts

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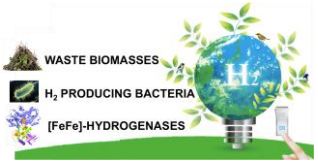

Bio-hydrogen ($bioH_2$) is a promising alternative energy to the existing fossil fuels [1]. Today, several bioreactors are based on the ability of various microorganisms to produce $bioH_2$ during dark fermentation from low cost waste materials [2]. One of the most fascinating challenges for future applications is the identification, characterisation and engineering of microorganism and of their enzymes involved in this process. The [FeFe]-hydrogenases (H_2ase) are key enzymes in the H_2 metabolism [4] and represent a promising source of inspiration for artificial catalysts for high efficiency H_2 production and in fuel cells [3, 4]. Oxygen (O_2) sensitivity is the main factor hindering the applicative exploitation of H_2ases as, generally, exposure to O_2 leads to irreversible inactivation in few seconds. Several hundreds of sequenced genes can potentially express enzymes of this class, but so far, only a few of them have been characterised. In this perspective the aims of this project are: *i*) Production of $bioH_2$ from low cost materials or waste biomasses by dark fermentation; *ii*) Isolation of H_2-producingbacteria; *iii*) Identification and characterisation of novel H_2ase.

i) Compost samples and the raw material of composting process (green agricultural wastes), were tested as feedstock for H_2 and CH_4 productions. The materials were supplied by AgriNewTech srl (co-funding the study with CRT/Lagrange Scholarships 2014-15). The compost samples or green wastes could produce H_2 and/or CH_4, without any pre-treatment or inoculum. The water amount played a key role in the process, as it can push towards the alternative production of H_2 or CH_4 [5].

ii) The culturable microflora from autumnal green wastes able to produce H_2 was isolated and characterised. Two highly efficient H_2 producers *Clostridium beijerinckii* AM2 and *Clostridium tyrobutyricum* AM6 were identified. *C. beijerinckii* has 6 H_2ase genes annotated, *C. tyrobutyricum* as only one reported. These H_2ase have not been studied yet. The spring green waste was enriched with *C. beijerinckii* AM2 and *C. tyrobutyricum* AM6. The correlation between $bioH_2$ production and H_2ase genes expression was studied by RT-qPCR. The results showed that *C. beijerinckii* AM2 prevailed over *C. tyrobutyricum* AM6 and a high expression modulation of the 6 different *C. beijerinckii* H_2ase genes occurred in the first 23 hours, suggesting a complex interplay of alternative pathways involving H_2ase with different time, and mode of expression.

iii) Five previously uncharacterised H_2ases from *C. beijerinckii* SM10 [6] (CbA5H, CbB3H, CbB2H, CbA8H) and *C. tyrobutyricum* AM6 (CtA2H) were cloned, expressed in *E.coli* [7] and tested by *in vivo* activity assays. CbA5H, CbB3H and CtA2H were readily expressed in active form. The most interesting was CbA5H, where we found that O_2 present in air does not cause any irreversible damage to the enzyme. The catalytic active state H_{ox} can switch to the O_2-insensitive inactive state H_{inact}. The transition is reversible and can be performed several times without any activity loss or spectral influence. The use of CbA5H (or engineered enzymes with similar properties) would make the exploitation in real applications much simpler and pave the way for highly efficient biocatalysts-based H_2-producing devices [8].

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High energy efficiency chemical processes for eco-friendly synthetic applications

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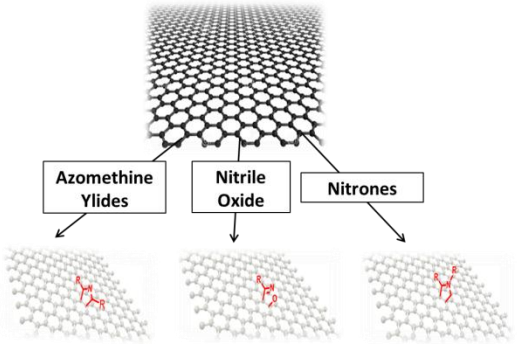
Chemical industries and laboratories are heavily involved in the development of mild, simple, environmentally friendly and inexpensive catalytic processes that adhere to the principles of green chemistry and process intensification, as well as fulfilling competitive production requirements. Currently, the huge gap between classic production processes and new protocols with green technologies, required a stronger interaction to find overlapping areas, in view of higher efficiency and sustainability. In this context we focused our attention in the development of green chemical procedures aimed to save energy, maximize the atom economy and increase process efficiency.

We exploited microwave (MW) irradiation and mechanochemical activation with planetary ball mills in green synthetic protocols. Besides excellent reaction yields these techniques enabled the replacement of organic solvents with benign reaction media, or even solventless.

We applied these new green procedures in different fields: 1) in the synthesis of fine chemicals and highly functionalized macromolecules; 2) in the preparation of new Pd-nanoparticles (NPs) catalyst on cyclodextrin/silica support; and 3) in the field of functionalization and grafting of carbonaceous materials.

Carbon-based nanomaterials have peculiar physical and chemical properties and their unique structure make them truly promising systems. In particular, the scientific community was inspired by graphene, a simple two-dimensional sheet of graphite with nano-size dimension, because of its large potential in different fields, from materials chemistry to drug delivery, imaging and therapy.[1] The surface modification of these carbon materials opens the possibility of tuning their properties in a controlled way. Based on our previous experience on covalent non-conventional functionalization of single-walled carbon nanotubes (SWCNTs)[2,3] and reduced graphene oxide (rGO)[4], we focus our efforts towards the environmentally friendly decoration of rGO. Three different approaches were compared based on 1,3-dipolar cycloaddition of azomethine ylides, nitrile oxide, and nitrones respectively. Real applications require a careful evaluation of functionalization degree by thermogravimetric analyses, FT-IR and Raman.



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Investigation on metabolites produced by lactic acid bacteria of food interest

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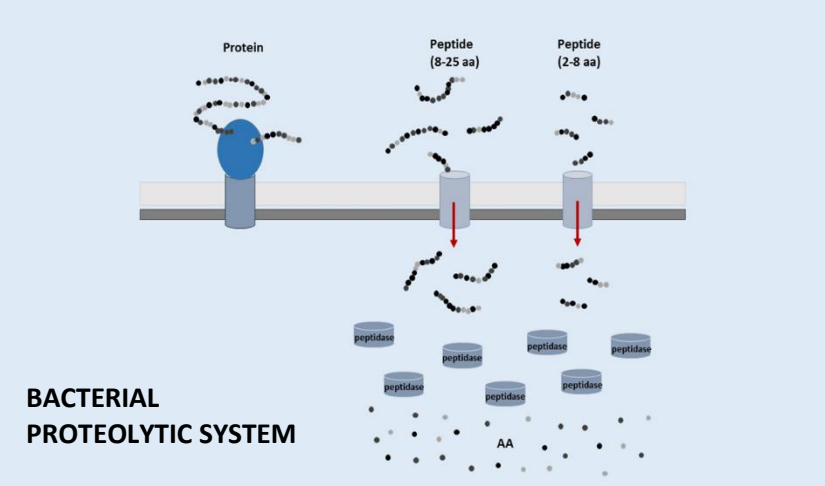
Tutor: Prof. Enrica Pessione

Recently, donkey milk has attracted increasing research interest due to its nutritional and functional properties (1). In fact, thanks to its peculiar composition, it can be considered a pharma-food/nutraceuticals product devoted to particularly sensitive categories of consumers (infants, seniors, allergic). Moreover, in Piedmont donkey breeding allows the preservation of environmental areas. The aim of this research project was to evaluate the ability of lactic acid bacteria (LAB) to improve the qualities of donkey milk in terms of bioactive peptide release. Milk proteins (especially caseins) represent the primary source of bioactive peptides, which can be encrypted within the amino acid sequence by bacterial proteolysis (2). The bioactive peptides exert several biological activities: immunomodulatory, antibacterial, antihypertensive, antioxidant, metal chelating and opioid-like. For these reason, the degradation of milk proteins by LAB proteolytic system is an attractive approach to generate functional foods enriched in bioactive peptides given the low cost and the positive nutritional image associated with fermented milk drinks and yogurt.
For this study, a pool of milk coming from donkeys with different ages and different stages of lactation has been used. In order to improve the freshness, the just milked milk has been lyophilized and reconstituted when required. A new method of pasteurization, consisting in a double pasteurization at different conditions, has been developed to guarantee sterility without compromising the matrix.
Five different LAB strains have been tested for their ability to grow in donkey milk. Among all, *L. rhamnosus* 17D10 and *L. lactis* subsp *cremoris* 40FEL3 have been selected for the best peptide production and fermentative power. The peptide mixture has been collected after 24 hours of fermentation, carried out by the two strains separately, and tested for the antimicrobial, antioxidant, ACE-inhibitor and metal-chelating activity. Furthermore, to characterize the peptides, present in the mixture, a MALDI-TOF MS analysis has been performed. The results obtained indicate that the employment of both *L. lactis* subsp *cremoris* and *L. rhamnosus* represents a good strategy to obtain a functionalized product, based on donkey milk, improved on its beneficial effects on human health.



LAB strain	Peptide (mg/ml)	pH
L. reuteri	0,082	5,73
L. helveticus	0,107	4,85
L. rhamnosus	0,283	3,56
L. cremoris	0,221	4.57
L. lactis	0,170	6,75

Resume table of the LAB strains tested for donkey milk fermentation and peptides production.

This research project was supported by Fondazione CRT

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Epidemiological and molecular study of pathogenicity characters of enterobacteriaceae isolated from humans and food

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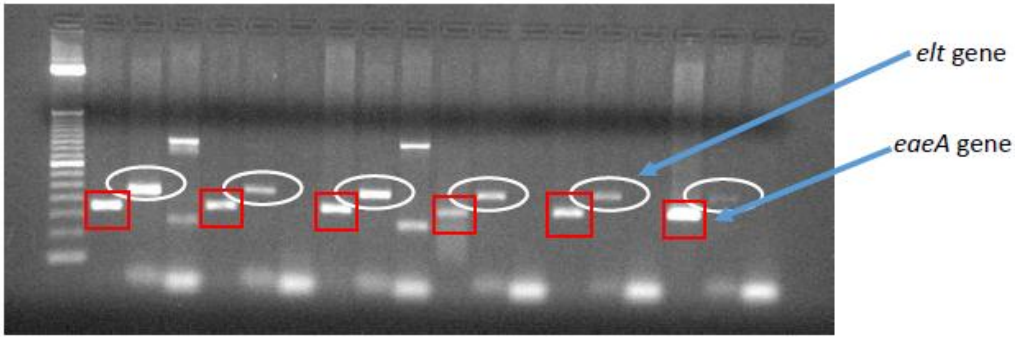
The human gastrointestinal tract represents one of the most densely populated microbial ecosystems detected to date. Although this microbial group has been recognized to have a crucial impact on human health, its precise composition in the first period of life is still subject to intense investigation.
The aim of the present case-control study was to describe the etiologic agents of diarrhea in infants, with special attention on diarrheagenic *E.coli* (DEC), the role of coinfections with other pathogens and the possible correlation between the presence of intestinal pathogens and risk factors such as age, feeding, nationality, sex and birth; other components of the infant gut microbiota (such as Lattobacilli, Bifidobacteria, Bacteroides) were investigated to better understand how individual differences can promote the multiplication of pathogens and how diarrhea influences gut health. Moreover another objective was to evaluate the possibility that early-life administration of probiotics (*Lactobacillus reuteri* DSM 17938) can modulate microbial composition in the gastrointestinal tract of infants, improving beneficial flora and reducing potential pathogens, and which modifications occurring in intestinal microbiota after antibiotic therapy. From March 2014 to July 2016 a total of 350 stool samples, from children aged between 0-6 months, were collected and examined, differently, for enteric pathogens (particularly DEC), Total aerobic counts, Total anaerobic gram-negative counts, Total anaerobic gram-positive counts, Enterobacteriaceae, Enterococci, Lactobacilli, Bifidobacteria. Viruses were the most frequently isolated agents in the diarrheal group, particularly Rotavirus (41.8%). Two categories of DEC were observed, precisely atypical enteropathogenic *E. coli* (a-EPEC 10% in case and 6.6% in control) and enterotoxigenic *E. coli* (ETEC 3.3% in case and 0.8% in control). About the effects of early-life administration of *Lactobacillus reuteri* DSM 17938 the two groups showed differences in gut microbial strains composition and richness. Infant treated with probiotics Lactobacilli count was significantly higher ($p < 0.05$). After 6 days of antibiotic treatment Lattobacilli e Bifidobatteri were no longer detected in the control group (without probiotics). The present research suggested that DEC may be an important and unrecognized cause of diarrhea in hospitalized infancy. The finding of diverse DEC types stressed the need for enhanced surveillance of gastroenteritis agents in infants with more active characterization of the *E. coli* isolated strains, since they are not routinarly detected in clinical practice. Moreover the study showed that fecal microbiota composition was significantly modified by antibiotic treatment and probiotic administration during or after antibiotic therapy can speed up the re-establishment of normal count of lactobacilli; early treatment with *L. reuteri* DSM 17938 in infancy can influence gut microbiota composition, improving gut health by reducing pathogen colonization. Further studies are needed to better understand how individual differences could act in the interaction between gut health and microbiota.



Representative gel showing multiplex PCR amplification of DNA extracted from E. coli strains

Advanced analytical approaches for "sensomic" investigations of high quality food matrices of vegetable origin

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Over the years, the importance of food quality evaluation has dramatically gained of importance because of the remarkable increase in the legal and consumers demand about safety, traceability and sensory impact (flavour and appearance). In this context, *Omics* is one of the approach of choice to evaluate food attributes. *Sensomics* focuses on analytical exertions to elucidate sensory-active compounds responsible for the multimodal perception (aroma, taste, texture, chemesthesis, etc) and aims at mapping the combinatorial code of aroma and taste-active key molecules (Chemical Odour and Taste Code) which generate the food perception. Multidimensional analytical platforms are often adopted in sensomics investigations to reach this objective, combining sample preparation-separation-detection-odour description-data mining. Comprehensive two-dimensional gas chromatography (GC×GC) coupled with Mass Spectrometric detection (MS) represent the most advanced GC platform for detailed odorant characterization.
In this perspective, the PhD project has been divided in different blocks concerning the development of the analytical platform, the characterization of food volatiles and the data processing.
A GC×GC platform equipped with parallel dual secondary column – dual detection (MS and FID) has been developed for the detailed analysis (identification and quantitation) of complex fractions of volatiles and semi-volatiles isolated and extracted from plants of food interest (tea, hazelnuts, cocoa) [1]. Furthermore, an innovative GC×GC platform based on microfluidic flow technology instead of cryogenic modulation has been experienced and efforts have been directed to the optimization of pattern matching procedures to enabling reliable methods translation between the two systems.
GC×GC platform has been used for the characterization of volatiles of food matrices of vegetable origin. In particular, the combination of different and complementary sampling approaches (Solid Phase Microextraction, SPME, Stir Bar Sorptive Extraction, SBSE, Head Space Sorptive Extraction, HSSE and Dynamic Headspace technique) with the separation power of GC×GC-MS, allowed to obtain information useful for fingerprinting and profiling studies of black tea volatiles (leaves and resulting infusions), considering that tea is a matrix of great interest for industry being one of the most consumed beverages worldwide.
Finally, different data processing tools have been adopted in the investigation of Extra Virgin Olive oils (EVOO) volatiles: in particular, *UT* (untargeted and targeted) fingerprinting approaches revealed to be consistent and reliable in defining optimal ripening indicators of olive fruits useful for olive oil classification [2].

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Innovative NMR bio-analytical applications

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The PhD project deals with the assessment of the high resolution NMR technique for the characterization of biological samples (analysis of metabolites in complex mixtures, quantification of metabolites and/or other molecules of interest in biological fluids). The project has been carried out by considering the four lines of activity described below.

Metabolic profiles of plasma samples enriched with growth factors: The metabolic analysis of plasma samples can be highly useful in determining the presence and degree of certain pathologies, and in following the response to treatment. A potential application is the monitoring of patients with myeloma treated with growth factors. We therefore decided to assess whether changes induced in plasma samples can be monitored by ^{1}H-NMR. We evaluated 80 samples of plasma (from 20 different patients), undergone to 4 different treatments. Spectra could indeed be classified in different groups, corresponding to the treatments, on the basis of the amounts of lactate, lipoproteins and alanine, which were identified by Principal Component Analysis (PCA) of the ^{1}H spectra. Thus, we can state that NMR associated to statistical analysis of the spectra is able to discriminate the groups of different samples by their metabolic profiles in a sufficiently accurate way.

Assessment of cell status in mesenchimal stem cells from dental pulp: Mesenchimal stem cells derived from dental pulp are a promising tool for bone regeneration. The NMR technique may be used to determine the stage of cell differentiation. We analyzed dental pulp stem cells (DPSCs) cell lisates at different stages of differentiation through ^{1}H and ^{31}P NMR. We found that the progression to mature cells can be related to different amounts of given metabolites such as choline and phospocholine. Thus, NMR in vitro can provide an efficient tool for analyzing the metabolism of dental pulp stem cells during the differentiation.

Determination of colon permeability by urine analysis after administration of sugars: NMR vs HPLC-MS
Sucralose is a sugar probe used for discrimination of site–specific alteration in grastrointestinal permeability. In the clinical practice, urine samples are analyzed by HPLC after oral administration of given amounts of sucralose to patients with suspected altered colon permeability: damaged colon membranes allow major amounts of sugar to pass them and to reach kidneys for excretion. Our aim was to assess the potentiality of NMR to quantify the amount of sucralose excreted in a DSS induced colitis mouse model. Urine collected from damaged mice and from a control group after oral administration of sucralose were analyzed by ^{1}H NMR and HPLC-MS. Both techniques are able to distinguish the DSS group from the healthy mice but HPLC-MS accuracy is limited at lower concentrations while NMR functions well in the whole concentration range.

LipoCEST for in vitro quantitative analysis: LipoCEST can be used to indirectly detect biomolecules of interest in a medium by looking at ^{1}H-the NMR signal of intraliposomal water. The NMR chemical shift (δ^{IL}) of water protons entrapped inside a vesicle filled with paramagnetic shift reagents (e.g. TmHPDO3A) can be fatherly increased by changing the vesicles shape. The frequency-encoding properties of asymmetrically shaped adducts has been exploited to develop an *in vitro* analytical assays for detecting the presence of enzymes and their activity. In particular, we investigated the effect of Matrix Metalloproteinase-2 (MMP-2) enzyme on biotinylated lipoCEST with streptavidin anchored on its surface. The cleavage by MMP2 of the bond between the lipoCEST and streptavidin leads to an overall change of LipoCEST/Streptavidin adduct architecture and consequently to a variation of LipoCEST δ^{IL}. The magnitude of δ^{IL} variation is proportional to the number of detached biotin/Streptavidin and therefore to the concentration of MMP-2. The technique can thus be used to quantify the enzyme activity.

Nano-sized systems for imaging, a focus on photoacoustic imaging

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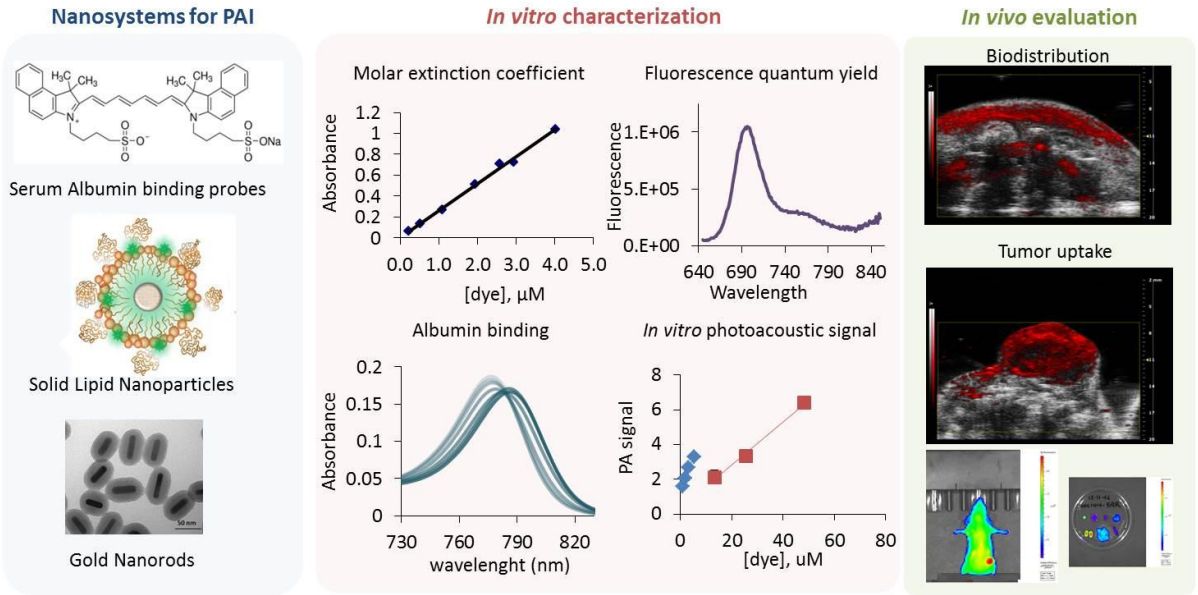
Tutors: Enzo Terreno, Alessandro Maiocchi

Photoacoustic imaging (PAI) is a new biomedical imaging modality based on laser-generated ultrasounds that allows the detection of endogenous and exogenous chromophores absorbing within the near-infrared wavelength range (NIR, ~600-1000 nm). Recently, PAI is applied to several biomedical areas including oncology, cardiovascular, neuroscience, gastroenterology, chronic inflammation, dermatology, to detect physiopathological processes in animal models and in human subjects during exploratory trials[1]. In order to explore the potentiality of new contrast agents for photoacoustic imaging, the *in vitro* characterization and *in vivo* application of some nano-sized probes is investigated. Nano-sized agents with long circulation times accumulate preferentially into tumor tissue due to the enhanced permeability and retention (EPR) effect[2]. However, it is known that this effect is heterogeneous among tumor types and within individual tumors. Therefore, the availability of an imaging tool that can report on the extent of the EPR effect could in principle improve the effectiveness of the administered therapy, particularly in the case of nanomedicines, providing additional insights on the response monitoring. We focus on different nano-sized systems with different dimensions for a detailed evaluation of their extravasation properties in tumor tissues. The nano-sized systems tested are: (i) a fluorescent small molecule with albumin binding properties, characterized by and average diameter of 5 nm; (ii) dye-loaded Solid Lipid Nanoparticles (SLNs, with a mean diameter of 45 nm); (iii) Gold Nanorods, non-spherical nanosystems with average dimensions: 40 nm x 10 nm. The main properties that influence the generation of photoacoustic signal, such as molar extinction coefficient, fluorescent quantum yield and albumin binding are investigated *in vitro*. Moreover, the photoacoustic signal is also measured by means of an agar phantom in different media such as phosphate buffer and serum. Based on the above parameters, a subset of nano-sized probes having enhanced photoacoustic signal is identified and their investigation is extended to healthy animals to confirm their *in vivo* efficiency. Near Infrared optical imaging is employed to characterize the biodistribution in the main organs and tumors of a subset of fluorescent probes.



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Functional characterization of the US12 gene family of Human Cytomegalovirus

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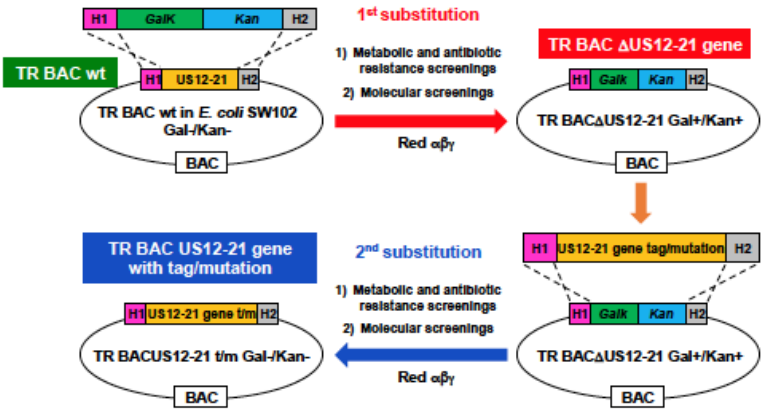
Human Cytomegalovirus (HCMV) is a widespread opportunistic viral pathogen that establishes a lifelong persistence in the host through both chronic and latent infections. HCMV causes life-threatening diseases in immunologically compromised individuals, such as transplant recipients and AIDS patients, for whom prolonged antiviral therapies are necessary. HCMV is also the major viral cause of congenital infections that lead to developmental abnormalities and fetal death (1-3). Nevertheless, to date, no vaccine is available to prevent HCMV infection and only a limited number of drugs all targeting the viral DNA polymerase are licensed to manage HCMV diseases. However, their clinical utility is limited by several drawbacks and no drugs have been yet approved for treatment of congenital HCMV infections. Given this, there is an urgent need to develop new, safe, and effective anti-HCMV compounds, possibly endowed with alternative mechanisms of action to avoid cross-resistance and decrease the risk of selection of resistant strains. Therefore, the identification and characterization of other HCMV proteins as virus-specific druggable targets is an essential step to design and develop new pharmacological strategies.

To this end, using a systematic reverse genetics approach by BAC-recombineering of the HCMV genome (summarized in the figure), we investigate the functional role of the US12 gene family of HCMV in the context of the viral replication in cell types relevant for viral pathogenesis, such as endothelial cells and epithelial cells. The US12 gene family is a set of 10 contiguous genes (US12 to US21) that constitutes about 5% of HCMV's genetic content and predicted to encode membrane-associated seven-transmembrane (7TMD) proteins. During my PhD, we have observed that: a) some US12 family members contribute to the HCMV cell tropism for specific cell types, as demonstrated by inactivation of US16, US18, US20, and US21 family members that abrogates virus growth in endothelial and epithelial cells; b) the US16 protein is required to regulate the entry of HCMV virions in these cell types (4); and c) the US20 protein is important in endothelial cells and regulates the composition of viral particles in a way as to influence a post-entry phase of the virus replication cycle in this cell type (5). Together, these findings characterize novel HCMV proteins as important factors for viral pathogenesis and whose functions could be exploited to design novel anti-HCMV intervention strategies.



Manipulation of HCMV US12-21 gene family by BAC recombineering

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Design, synthesis and pharmacological characterization of new chemical entities targeting NLRP3 inflammasome activation and related signaling pathways

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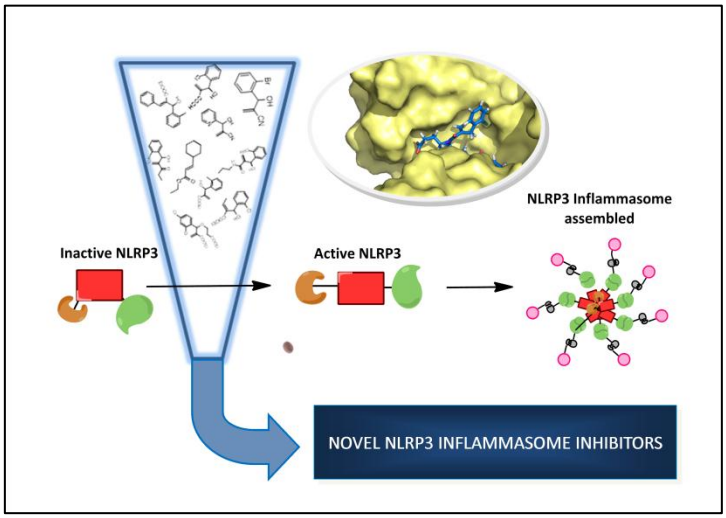
NLRP3 inflammasome is a multiprotein complex that plays a crucial role in activating caspase-1, processing the pro-inflammatory interleukin-1β (IL-1β), and triggering pyroptotic cell death cascade.[1] Gain of function mutations in NLRP3 determine its abnormal activation which is a key factor in the pathogenesis of autoinflammatory diseases known as cryopyrin-associated periodic syndromes (CAPS). The progression of other diseases, such as atherosclerosis, Inflammatory Bowel Disease (IBD), type-2 diabetes, Alzheimer's disease, and gout is also dependent on NLRP3 inflammasome activation.[2] So far, several strategies have been proposed to dampen NLRP3 activity, among them covalent drug development seems to be a promising one.[3]
In this project we designed and synthesized lead compounds acting either as direct NLRP3 inhibitors or as multi-target agents acting multiple stages of NLRP3 signaling pathway. Synthesis of "*ad hoc*" designed chemical probes was also considered to investigate mechanism of NLRP3 activation. The work was articulated in three steps: synthesis of covalent drugs directly targeting NLRP3 (first year)[3]; modulation of cytotoxic properties of electrophilic warheads towards safer compounds (second year)[4]; increase of specificity towards NLRP3 ATPase pocket, investigation of mechanism of action and exploration of other key players of the pathway (third year).
In this work, we successfully identified new candidates for NLRP3 inflammasome inhibition characterized by good in vitro anti-pyroptotic activity, optimized toxicological profile, direct NLRP3 ATPase inhibition, high ability to decrease in vitro and in vivo IL-1β production. Selected compounds showed promising activities in pharmacological models of CAPS,[4] ischemia,[5] and IBD[6].



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Multiparametric MRI studies in murine tumor models

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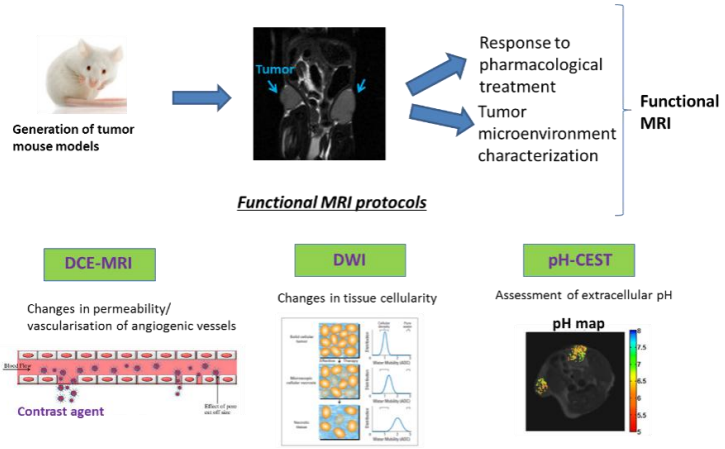
Tumor microenvironment properties as vascularization, hypoxia, metabolism and acidosis play a fundamental role in the tumor progression and metastasis formation process. These features can be non-invasively investigated as alternative tumor biomarkers by functional imaging approaches. The aim of this PhD project involves the implementation of multimodal protocols to characterize tumor microenvironment and evaluate early response to pharmacological treatments in several tumor murine models. The dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) technique was used to quantify the tumor vascularization properties by exploiting a dedicated preclinical gadolinium-blood pool contrast agent. It was developed to accurately assess tumor vessels perfusion and permeability, owing to its specific extravasation only in presence of tumor leaky vasculature, in comparison to clinical small molecular contrast agents. In addition, the diffusion weighted imaging (DWI) technique was investigated to obtain information about tissue cellularity changes. Moreover, a quantitative clustering approach was developed to assess tumor heterogeneity and changes in the evaluation of the response to pharmacological treatments. A remarkable point relies on the combination of these techniques with low magnetic field scanner, facilitating the translation of these approaches in the clinical settings. An additional MRI approach was developed to detect contrast enhancement properties generated by several iodinated contrast agents that are clinically approved for computed tomography (CT). Their capability to quantify tumor perfusion and vascularization properties with the proposed chemical exchange saturation transfer (CEST) MRI approach were compared to the information obtained by CT. In addition, the potential of iodinated as perfusion agents for MRI applications were compared to contrast enhancement and perfusion estimates provided by conventional gadolinium ones. Furthermore, iodinated contrast agents are indicated as responsive of extracellular pH in tissue, which is reported to be particularly acidic in tumor as a consequence of its altered glycolytic pathways. This relation between acidosis and abnormal glucose consumption in tumor was investigated by comparing extracellular pH measurement by the MRI-CEST approach with 18F-FDG uptake measured by positron emission tomography (PET) in a murine tumor model. Moreover, changes in tumor acidosis were investigated to monitor the tumor progression and the response to pharmacological treatment.
The combination of these functional MRI techniques were in particular implemented for the characterization and treatment monitoring of gastrointestinal stromal tumor (GIST) xenografts sensitive and resistant to the first line treatment imatinib. For this purpose, imatinib sensitive and resistant GIST xenograft mouse models were generated and characterized by DCE-MRI, DWI and MRI MRI-pH CEST modalities. Secondly, the multiparametric MRI approach was combined with PET technique to evaluate early therapeutic response to imatinib treatment.



Role of 4-methyl sterols in post-squalene metabolic diseases and cancers Hedgeogh protein pathway depending

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The aims of this project are to characterize the enzymes of the steroid-C4-demethylase complex and to evaluate the role of 4-methyl-sterols in the Hedgehog (*Hh*) protein pathway, a signalling cascade involved in the embryogenesis, in post-squalene metabolic and cancer diseases.
Firstly, a fermentative method producing 4-methylzymosterone, substrate of an enzyme (HSD17B7) of the demethylase complex and probably correlated to a defect of the Hh signalling pathway activation, was developed starting from yeast strains engineered in the *ERG27* (3-ketosteroid reductase) gene. Next, compound was fully characterized by spectroscopic (NMR) analysis, and a second product, 4-methyl fecosterone was isolated and characterized. The two 4-methylsterones were tested on two different cell lines and the acquired preliminary data showed an anti-proliferative activity of the two molecules Therefore, in collaboration with the Turku Center for Disease Modeling in Finland, we tested the molecules activity *in vivo* on pregnant murine models to verify the supposed direct association between 4-methylzymosterone accumulation and embryo malformations [Barsh et al., 2011]. The preliminary results acquired show some interesting morphological and histological differences between control and treated groups.
A method to produce a mixture (named Keto 1 fraction) of the above 4-methyl sterones in the radioactive form was also developed, in order to use them as substrates in enzymatic characterization of C4-demethylase complex. To better characterize the mammalian HSD17B7 (the 3-ketosteroid reductase belonging to the C4-demethylase complex), human enzyme was expressed in *E. coli* cells. Comparative experiments were carried out with the yeast orthologue ERG27 enzyme expressed in *E. coli* cells too.
Experiments with human and yeast recombinant enzymes incubated with radioactive 4-methyl-zymosterone, as sterol substrate, revealed that both are highly active, thus demonstrating that they do not need the presence of the other proteins of the complex. Assays with radioactive estrone, as steroid substrate, showed that the human enzyme possesses an intrinsically high estrogenic activity outside of its cellular/tissue environment, contrary to the yeast enzyme in which estrogenic activity is completely absent.
Once we obtained these results using the radioactive substrates, we started to investigate deeper into the intrinsic double reduction activity of HSD17B7 in two different ways. (i) Using the non-radioactive substrates, more available than the radioactive one, we tested, on both the activities, different inhibitors, already proved to be active on the estrone reduction activity [Bellavance et al.,2009]. Surprisingly, at the same experimental conditions, they inhibited all the steroid reduction activities contrary to the sterol one, which was not affected even by high inhibitors concentrations (100 μM). These evidences posed more interesting questions about the enzyme structure-activity relationship. (ii) Therefore, a period was spent abroad, at the King's College of London, to design and produce different HSD17B7 point-mutated versions. Some mutants showed differences between the steroid reduction activity end the sterol one. Moreover, in the same period, we undertake different strategies to study and increase the solubility of this membrane enzyme, for a crystallization purpose; a promising result was obtained using an oxidative refolding process.
To enlarge the collection of intermediates to be used as substrates of C4-demethylase complex and/or inhibitors of the Hedgehog proteins, radiolabelled and non-radiolabelled 4-methylsterones were treated with $NaBH_4$ to produce 3-hydroxy derivatives. The two radioactive isomers (3-beta, 3-alfa) produced by chemical reduction were isolated and used as substrates in preliminary assays with cell homogenates from wild-type *S. cerevisiae* strain (SCY876). Results showed that the yeast demethylase complex had a marked preference for the beta-isomer (68% vs. 13% transformation). This evidence points out a high stereoselectivity for the enzymes of C-4 demethylase complex suggesting that the "bad substrate" alpha isomer could be used as inhibitor.

Liposomes for the active targeting of anticancer drugs against receptors overexpressed on cancer stem cells for the treatment of pancreatic adenocarcinoma

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Pancreatic adenocarcinoma (PDAC) is one of the most aggressive and devastating human malignancies with a death-to-incidence ratio of 0.99 and most of the patients presenting with metastatic disease at the time of diagnosis. More than 75% of patients who undergo surgical resection of small pancreatic tumors with clear surgical margins and no evidence of metastasis, die from metastasis within 5 years.
This strange and complicated clinical feature is related to the presence of particular type of cancer cells called cancer stem cells (CSC) (1). CSC are a small population of cancer cells, within the tumor, highly tumorigenic, resistant to conventional therapy and able to replicate cancer when transplanted in mice. Currently pancreatic CSC are believed responsible for cancer recurrence and resistance and represent a new target in PDAC therapy.
A promising approach to eradicate pancreatic CSC is active targeting. Using this strategy, drugs encapsulated in nanosystems and decorated with opportune carriers, should be delivered in a specific manner on CSC by recognition of receptors expressed on their surface. In this work, the active targeting approach was reached using liposomes decorated with hyaluronic acid (HA), containing disulfiram (DSF) and disulfiram-copper complex (DSF-CU).
This system, HA-liposomes-DSF, can represent a "bullet" to kill CSC. It is well known, indeed, that DSF and its copper complex are potent inhibitors of CSC (2) while HA is a natural ligand of CD44 receptor which is considered a surface marker of pancreatic CSC.
To prepare HA-liposomes we initially synthesized a conjugate between HA and a phospholipid (HA-DPPE) that was added during liposomes preparation. In this manner we obtained liposomes with HA functionalized surface. Liposomes were then characterized in terms of size, Z potential, drugs concentration, HA density and stability in stock and physiological conditions. To investigate the interactions between DSF and the liposomes membrane, DSC analysis were performed.
Then, the cellular uptake of fluorescent HA-liposomes on CD44 positive pancreatic cancer cell lines(PANC-1) was evaluated. We observed an increasing uptake for hyaluronated formulations in comparison to plain ones, suggesting a receptor mediated uptake.
Finally, liposomal formulation were tested*in vitro* on PANC-1 cell lines, either on parental or CSC models. Liposomal formulations showed stronger anticancer activity in comparison to the free compounds. Moreover,HA-liposomes containing DSF-Cu were tested versus plain formulation and a further decrease on cells viability was observed.

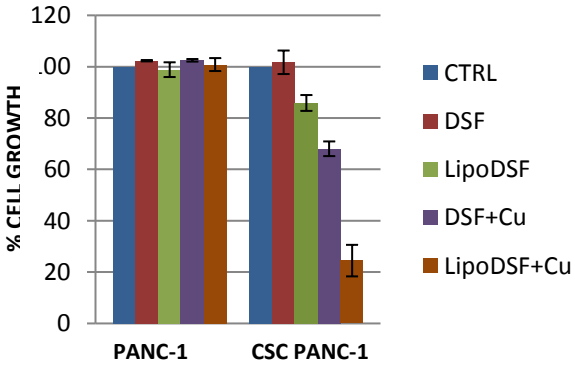

Fig 1: Cytotoxic activity of DSF and DSF-Cu liposomes on PANC-1 cells (parental and CSC) at 72 h and 100 nM.

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New nanosized agents for imaging guided treatment of tumours

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Personalized medicine is defined, according to the U.S. National Institutes of Health (NIH), as 'a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease' [1]. The ultimate goal of this medicine is to furnish the proper treatment to the right person at right time, with potential benefits including increased response rates and survival, as well as reduced toxicity [1]. The impact of personalized medicine is contingent upon a systematic discovery of novel biomarkers that can be prognostic and predictive for patient outcome. It is well known that quantitative and selective biomarker detection and rapid profiling of diseased cells in unprocessed biological samples (biopsies, blood, needle and aspirate) is the key-point of this type of therapy. The urgent need is to take a step forward from the simple diagnosis of specific receptor positivity to a stratification of patients based on a quantitative estimation for seeking an improved matching to the responsiveness to one of the available targeted therapy. The available methods, mainly based on histological slide-tests, do not provide an adequate response to this medical need as they suffer for a poor reproducibility and a large heterogeneity in the regions of the tissue specimen [2]. In this context, a new diagnostic method, named R-ELISA (Relaxometric Enzyme Linked in Cells Suspension Assay) has been proposed in this PhD project, for the quantification of biomarkers of interest based on the combination of Nuclear Magnetic Resonance (NMR) relaxometric technology with an enzymatic amplification procedure, detecting the target epitopes in a way comparable to techniques like ELISA. This *in vitro* diagnostic method is based on the release of paramagnetic species from relaxometrically "silent" liposomes operated by the action of a phospholipase A_2 (PLA_2), previously targeted to the epitope of interest. The released paramagnetic species causes an increase of the longitudinal water proton relaxation rate proportional to the number of PLA_2 bound to the cell outer surface. The sensitivity of the herein proposed method, was attempted in the detection of folate receptor expression on human ovarian cancer cells by functionalizing PLA_2 with folic acid and validated by well-established spectrofluorimetric procedures [3]. In addition, for "in vivo" evaluation of biomarkers expression and "in real time" drug biodistribution, different nanoparticles (PLGA-NPs) have been proposed in this project, both based on use of PolyLactic and Glycolic Acid polymer and suitable for imaging guided drug delivery. Firstly, because the magnetic iron oxide nanoparticles (Fe-NPs) can be exploited in biomedicine as agents for magnetic fluid hyperthermia (MFH) treatments and as contrast enhancers in magnetic resonance imaging, new oleate-covered Fe-NPs have been prepared (either by co-precipitation or thermal decomposition methods) and incorporated into PLGA-NPs (PLGA-Fe-NPs) to improve their biocompatibility and in vivo stability. The NPs formulations are characterized by peculiar ^{1}H nuclear magnetic relaxation dispersion (NMRD) profiles that directly correlate with their heating potential when exposed to an alternating magnetic field. Furthermore, the PLGA-Fe-NPs have been loaded with paclitaxel to pursue an MFH-triggered drug release. By prolonging the magnetic field exposure to 30 min, a significant drug release was observed for PLGA-Fe-NPs in the case of the larger-sized magnetic NPs. [4]. Secondly, PLGA-NPs, coated with L-Ferritin, have been exploited for the simultaneous delivery of paclitaxel and an amphiphilic Gd based MRI contrast agent into breast cancer cells (MCF7). Ferritin moieties endow PLGA-NPs with targeting capability, exploiting SCARA5 receptors overexpressed by these tumour cells, that results in an increased paclitaxel cytotoxicity. Moreover, protein coating increased NPs stability thus reducing the fast and aspecific drug release before reaching the target. The theranostic potentiality of the NPs has been demonstrated by evaluating the signal enhancement on T1-weighted MRI images of labelled MCF7 cells, shown a good capability to target cancer cell line with higher level of SCARA5 receptors[5].

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Targeting the *human* Dihydroorotate Dehydrogenase (*h*DHODH) by a Scaffold Hopping Bioisosteric approach using Hydroxy-azoles

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The human isoform of the dihydroorotate dehydrogenase enzyme (hDHODH) catalyses the fourth step of the de novo pyrimidine synthesis, a biosynthetic pathway enhanced in proliferating cells such as activated T-lymphocytes, and cancer cells. The efficacy of hDHODH inhibitors in the treatment of rheumatoid arthritis and multiple sclerosis has been evaluated, and leflunomide (Arava®) and its active metabolite teriflunomide (Aubagio®) have been already approved for therapy. Brequinar is another well-studied hDHODH inhibitor, but it was unsuccessfully evaluated against a number of tumour categories due to several drug-related side effects.[1]
Inside the inhibitor-binding site, conventionally divided into five subsites, two different binding-modes have been described in literature.[2]. Starting from structural information from both brequinar and teriflunomide, a scaffold hopping approach was applied using hydroxy-azoles system, affording a new series of products able to establish additional interactions with subsites **3** and **4**. The general model of these compounds (**1**) is a hydroxylated heterocycle linked to a biphenyl system through an amide bond.[3] According to molecular modelling studies, the deprotonated acidic moiety should interact with residues Arg**136** and Gln**47** of subsite **2** (Brequinar-like binding-mode[2]), while the biphenyl system may establish lipophilic interactions with residues of subsites **1** and **5** (*Figure* **a**). New compounds showed inhibitory activity on recombinant *h*DHODH with IC_{50} values in the nanomolar range and inhibition of human T-cell proliferation comparable to brequinar and teriflunomide. Our theoretical design, modelling, synthesis, SAR, X-Rays and biological assays, as well as cell viability, proliferation, cytotoxicity and immunosuppression results are presented in detail.

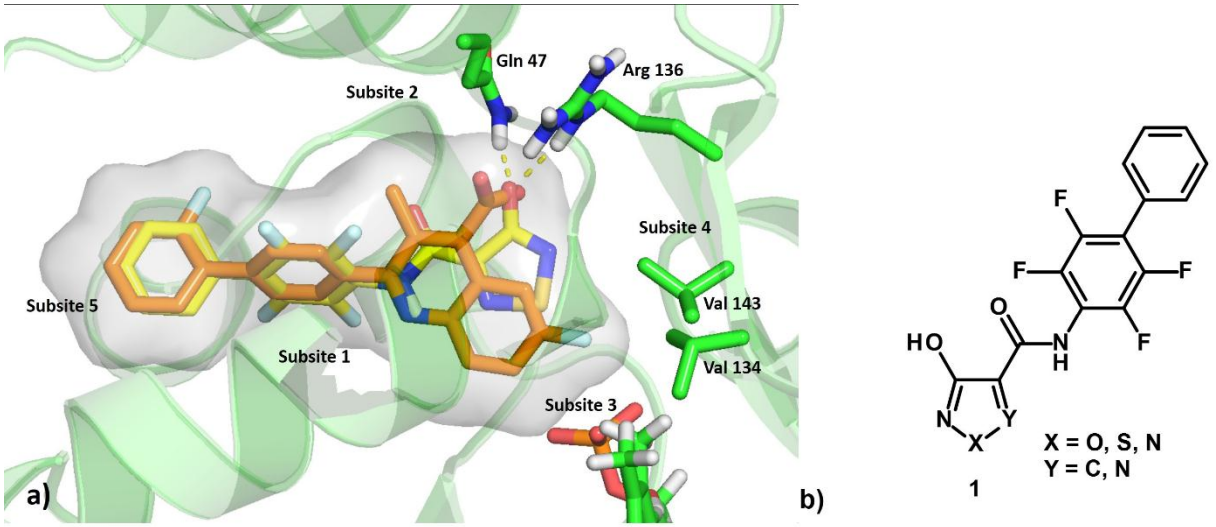

Fig. 1. a) The lipophilic patch of the hDHODH binding site in complex with brequinar (green) and our best compound (yellow). b) General structure of new inhibitors (1).

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Progetti di ricerca con conclusione nel 2017, 2018 e 2019

(cicli 30°, 31° e 32°)

Progetti di ricerca attivi nel triennio 2014-2017
(30° ciclo di Dottorato)

	Nominativi	Progetto	Struttura/e di riferimento*
Dottoranda Tutor	Chiara Agliassa Massimo Maffei (massimo.maffei@unito.it)	Effects of Earth's magnetic field on plant growth, development and evolution	Dipartimento di Scienze della Vita e Biologia dei Sistemi
Dottoranda Tutor	Federica Bosca Alessandro Barge (alessandro.barge@unito.it)	Synthesis, characterization and structure-property relationships studies of molecular and supramolecular systems for sonodynamic therapy applications	Dipartimento di Scienza e Tecnologia del Farmaco
Dottorando Tutor	Davide Bressanello Erica Liberto (erica.liberto@unito.it)	Correlative studies between sensory characterization and chemical composition of food matrices of different composition and origin	Dipartimento di Scienza e Tecnologia del Farmaco; Progetto finanziato da Lavazza
Dottoranda Tutor	Eleonora Cavallari Francesca Reineri (francesca.reineri@unito.it)	Novel hyperpolarized probes for the development of metabolic imaging	Dipartimento di Biotecnologie Molecolari e Scienze per la Salute
Dottoranda Tutor	Benedetta Ferrara Roberto Fantozzi (roberto.fantozzi@unito.it)	Development of immuno-mediated pharmacotherapy models for the treatment of melanoma	Dipartimento di Scienza e Tecnologia del Farmaco
Dottorando Tutor	Alessandro Giraudo Marco Lolli (marco.lolli@unito.it)	Development of innovative GABAA analogues using a bioisosteric approach	Dipartimento di Scienza e Tecnologia del Farmaco, in co-tutela con il Department of Drug Design and Pharmacology, University of Copenhagen
Dottorando Tutors	Riccardo Leinardi Francesco Turci (francesco.turci@unito.it) Gianmario.Martra (gianmario.martra@unito.it)	Interfacial molecular recognition in complex systems: chemical investigation of the interaction of oxide surfaces and cell membrane	Dipartimento di Chimica
Dottoranda Tutors	Luisa Mandrile Andrea Mario Rossi (a.rossi@inrim.it) Gianmario Martra (gianmario.martra@unito.it)	Application of vibrational spectroscopy to the detection of biomolecules of interest in food metrology	Dipartimento di Chimica; progetto in collaborazione con l'Istituto Nazionale di Ricerca Metrologica (INRIM)
Dottorando Tutors	Amerigo Pagoto Silvio Aime (silvio.aime@unito.it) Enzo Terreno (enzo.terreno@unito.it)	MRI/Optical imaging agents to target biomarkers of prostate adenocarcinoma	Dipartimento di Biotecnologie Molecolari e Scienze per la Salute; con il supporto dell'Associazione Italiana Ricerca sul Cancro
Dottoranda Tutor	Roberta Tassinari Roberto Bono (roberto.bono@unito.it)	Life and occupational environment, lifestyles and physio-pathological conditions of human populations. An epidemiological study of oxidative stress and health effects to design the best policies of primary prevention.	Dipartimento di Scienze della Sanità Pubblica e Pediatriche
Dottoranda Tutor	Giulia Trucco Roberto Bono (roberto.bono@unito.it)	Oxidative damage in workers exposed to wood dust. The mechanism of action of this carcinogen according to an innovative approach of the molecular epidemiology	Dipartimento di Scienze della Sanità Pubblica e Pediatriche; con il supporto di INAIL, Sezione Piemonte

* per informazioni su Dipartimenti e Gruppi di Ricerca vedere la sezione successiva

Progetti di ricerca attivi nel triennio 2015-2018
(31° ciclo di Dottorato)

	Nominativi	Progetto	Struttura/e di riferimento*
Dottoranda Tutors	Annasofia Anemone Walter Dastrù (water.dastru@unito.it) Silvio Aime (silvio.aime@unito.it)	Mapping pH in the tumour extracellular region as new MRI biomarker in oncology	Dipartimento di Biotecnologie Molecolari e Scienze per la Salute (DBMSS)
Dottoranda Tutor	Francesca Barbero Cinzia Bertea (cinzia.bertea@unito.it)	Chemical signaling in multitrophic interactions involving plants and insects	Dipartimento di Scienze della Vita e Biologia dei Sistemi (DBIOS)
Dottoranda Tutor	Irene Maria Carnovale Marco Lolli (marco.lolli@unito.it)	Synthesis of new Gadolinium-based contrast agents (GBCAs) for MRI imaging	Dipartimento di Scienza e Tecnologia del Farmaco; progetto finanziato da Bracco Imaging Spa
Dottorando Tutor	Alberto Ciaramella Gianfranco Gilardi (gianfranco.gilardi@unito.it)	Structural and Functional Characterisation of CYP116B5: a new class VII catalytically self-sufficient bacterial P450	Dipartimento di Biotecnologie Molecolari e Scienze per la Salute
Dottoranda Tutor	Alessia Cordaro Enzo Terreno (enzo.terreno@unito.it)	Characterization of fluorescent probes for imaging guided surgery	Dottorato in Apprendistato presso Bracco Imaging Spa; Dipartimento di riferimento: DBMSS
Dottorando Tutor	Gao Chongliang Sheila Sadeghi (sheila.sadeghi@unito.it)	Site-directed mutagenesis studies of human flavin-containing monooxygenase 3	Dipartimento di Scienze della Vita e Biologia dei Sistemi
Dottorando Tutors	Giuseppe Mannino Maffei Massimo (massimo.maffei@unito.it) Andrea Occhipinti (andrea.occhipinti@unito.it)	Chemical analyses and extraction techniques for quality control of food and dietary supplements	Dottorato in Apprendistato presso Biosfered; Dipartimento di riferimento: DBIOS
Dottoranda Tutors	Laura Rotolo Giancarlo Cravotto (giancarlo.cravotto@unito.it)	Enabling technologies for clean and sustainable synthetic processes	Dipartimento di Scienza e Tecnologia del Farmaco
Dottoranda Tutor	Federica Sodano Loretta Lazzarato (loretta.lazzarato@unito.it)	Photoinduced nitric oxide selective release in mitochondria	Dipartimento di Scienza e Tecnologia del Farmaco

* per informazioni su Dipartimenti e Gruppi di Ricerca vedere la sezione successiva

Progetti di ricerca attivi nel triennio 2016-2019
(32° ciclo di Dottorato)

	Nominativi	Progetto	Struttura/e di riferimento*
Dottorando Tutors	Stefano Acquadro Patrizia Rubiolo (patrizia.rubiolo@unito.it)	Isolation and characterization of plant extracts of pharmaceutical, cosmetic and food interest by bioassay guided studies	Dipartimento di Scienza e Tecnologia del Farmaco
Dottoranda Tutor	Federica Bessone Roberta cavalli (roberta.cavalli@unito.it)	Multifunctional oxygen-filled nanobubbles for the treatment of wound healing	Dipartimento di Scienza e Tecnologia del Farmaco
Dottorando Tutor	Bonanni Davide Marco Lolli (marco.lolli@unito.it)	In silico evaluation of drug design innovative bioisosteric replacement	Dipartimento di Scienza e Tecnologia del Farmaco
Dottoranda Tutor	Cristina Campobenedetto Cinzia Bertea (cinzia.bertea@unito.it)	Chemical characterization of new products with biostimulant action and study of their effects on plant growth and development by using genomic and metabolomic techniques	Dottorato in Apprendistato presso Green Has; Dipartimento di riferimento: DBIOS
Dottorando Tutor	Federico Capuana Silvio Aime (silvio.aime@unito.it)	Design and testing of novel responsive imaging probes	Dipartimento di Biotecnologie Molecolari e Scienze per la Salute; con il supporto del progetto EU-H2020 Single Photon Counting CT
Dottoranda Tutor	Marta Rosso Cialié Chiara Cordero (chiara.cordero@unito.it)	Advanced analytical approaches for “omic” investigations of high quality food matrices of vegetable origin	Dipartimento di Scienza e Tecnologia del Farmaco; progetto finanziato da Ferrero
Dottorando Tutor	Michele Durante Maffei Massimo (massimo.maffei@unito.it)	Sunflower varieties for the food industry	Dipartimento di Scienze della Vita e Biologia dei Sistemi; con il supporto di So.Re.MO
Dottoranda Tutors	Federica Galati Giorgio Gribaudo (giorgio.gribaudo@unito.it)	Characterization of 7TMD proteins of Herpesvirus: from genes to functions	Dipartimento di Scienze della Vita e Biologia dei Sistemi
Dottoranda Tutor	Ge Xinyu Giancarlo Cravotto (giancarlo.cravotto@unito.it)	Innovative technologies for the recovery, adsorption or degradation of pharmaceuticals and other persistent organic pollutants from industrial or urban waste water	Dipartimento di Scienza e Tecnologia del Farmaco
Dottorando Tutor	Giorgio Grillo Giancarlo Cravotto (giancarlo.cravotto@unito.it)	Design of non-conventional batch and flow chemical processes for biomass valorization	Dipartimento di Scienza e Tecnologia del Farmaco
Dottorando Tutor	Monirul Islam Maffei Massimo (massimo.maffei@unito.it)	Plant magnetoreception: funcional role, molecular biology and physiology of cryptochrome	Dipartimento di Scienze della Vita e Biologia dei Sistemi

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Progetti di ricerca attivi nel triennio 2016-2019
(32° ciclo di Dottorato)

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	Nominativi	Progetto	Struttura/e di riferimento*
Dottoranda Tutors	Francesca La Cava Francesca Reineri (francesca.reineri@unito.it)	Hyperpolarized contract agents for in vivo MRS-MRI applications using Para-Hydrogen	Dipartimento di Biotecnologie Molecolari e Scienze per la Salute; progetto finanziato da Bracco Imaging Spa
Dottoranda Tutors	Maria Jesús Morán Plata Giancarlo Cravotto (giancarlo.cravotto@unito.it)	Combining ultrasound and microwaves in chemical processes	Dipartimento di Scienza e Tecnologia del Farmaco; dottorato svolto nell'ambito del progetto H2020 COSMIC
Dottorando Tutors	Stefano Nebbia Enrica Pessione (enrica.pessione@unito.it) Laura Cavallarin (laura.cavallarin@ispa.cnr.it)	Effect of food processing on protein structure, functionality and allergenicity	Dipartimento di Scienze della Vita e Biologia dei Sistemi; progetto finanziato da CNR-ISPA
Dottoranda Tutors	Ana Luisa Sotuelo Giancarlo Cravotto (giancarlo.cravotto@unito.it)	Ultrasound- and/or microwave-assisted C=C bond activation	Dipartimento di Scienza e Tecnologia del Farmaco; dottorato svolto nell'ambito del progetto H2020 COSMIC
Dottorando Tutor	Ivano Vigliante Andrea Occhipinti (andrea.occhipinti@unito.it)	Chemical analysis and food processing of sunflower natural extracts	Dipartimento di Scienze della Vita e Biologia dei Sistemi; progetto finanziato da So.Re.MO

* per informazioni su Dipartimenti e Gruppi di Ricerca vedere la sezione successiva

Dipartimenti e Gruppi di Ricerca afferenti al Dottorato

DIPARTIMENTO DI BIOTECNOLOGIE MOLECOLARI E SCIENZE PER LA SALUTE

www.dbmss.unito.it

Componenti del Collegio dei Docenti (CD) e Tutor (T) del Dottorato afferenti a questo Dipartimento appartengono ai gruppi:

Computer-Assisted Strategies and Synthesis in Medicinal Chemistry

www.casmedchem.unito.it

componente del gruppo afferente al Dottorato

nominativo	contatto
Prof.ssa Giulia Caron (T)	**giulia.caron@unito.it**

Molecular Imaging

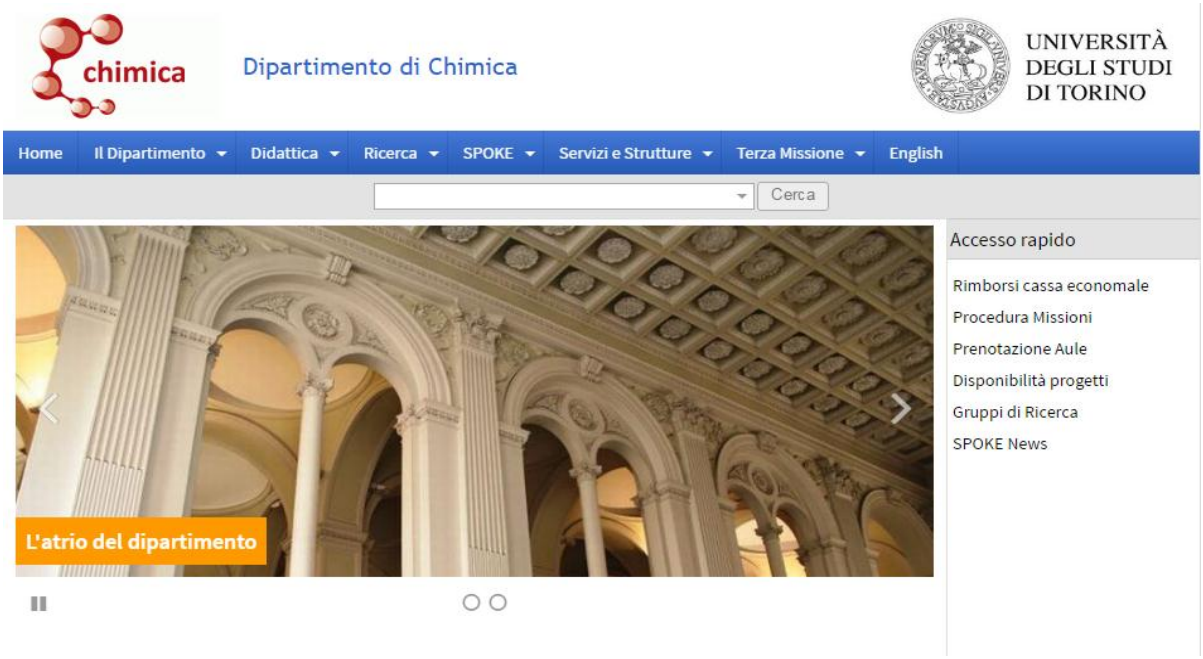
http://www.dmbhs.unito.it/do/home.pl/View?doc=Aime_group.html

componenti del gruppo afferenti al Dottorato

nominativo	contatto
Prof. Silvio Aime (CD)	**silvio.aime@unito.it**
Dott. Walter Dastrù (T)	**walter.dastru@unito.it**
Dott.ssa Daniela Delli Castelli (CD)	**daniela.dellicastelli@unito.it**
Dott.ssa Francesca Reineri (T)	**francesca.reineri@unito.it**
Prof. Enzo Terreno (CD)	**enzo.terreno@unito.it**

DIPARTIMENTO DI CHIMICA

www.chimica.unito.it



I componenti del Collegio dei Docenti del Dottorato afferenti a questo Dipartimento appartengono ai gruppi:

FABLAB - Forensic, Analytical & Bioanalytical Laboratories

http://www.chimica.unito.it/do/gruppi.pl/Show?_id=du5p

componente del gruppo afferente al Dottorato	
nominativo	**contatto**
Prof.ssa Cristina Giovannoli	**cristina.giovannoli@unito.it**

SURFIN – Surface and Interface Physical Chemistry

http://www.chimica.unito.it/do/gruppi.pl/Show?_id=cpk9

componente del gruppo afferente al Dottorato	
nominativo	**contatto**
Prof. Gianmario Martra	**gianmario.martra@unito.it**

DIPARTIMENTO DI SCIENZA E TECNOLOGIA DEL FARMACO

www.dstf.unito.it

Componenti del Collegio dei Docenti (CD) e Tutor (T) del Dottorato afferenti a questo Dipartimento appartengono ai gruppi:

Biochemistry and Molecular Biology (BMB)

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=7tlj

componente del gruppo afferente al Dottorato

nominativo	contatto
Prof.Gianni Balliano (T)	gianni.balliano@unito.it

Medicinal Chemistry -Group MC2

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=8b36

componenti del gruppo afferenti al Dottorato

nominativo	contatto
Prof. Massimo Bertinaria (T)	massimo.bertinaria@unito.it
Dott. Marco Lolli (T)	marco.lolli@unito.it

Medicinal Chemistry - Group NOPhArm (DRUG DESIGN- NO Prodrugs and hybrids)

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=o9ik

componenti del gruppo afferenti al Dottorato

nominativo	contatto
Dott. Konstantin Chegaev (T)	konstantin.chegaev @unito.it
Prof.ssa Loretta Lazzarato (CD)	loretta.lazzarato@unito.it

Organic Chemistry

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=8zud

componenti del gruppo afferenti al Dottorato

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Dott. Alessandro Barge (T)	alessandro.barge @unito.it
Prof. Giancarlo Cravotto (CD)	giancarolo.cravotto@unito.it
Dott. Silvia Tagliapietra (T)	silvia.tagliapietra@unito.it

Pharmaceutical Biology and Food Chemistry

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=82xu

componenti del gruppo afferenti al Dottorato

nominativo	contatto
Prof.ssa Chiara Cordero (CD)	chiara.cordero@unito.it
Dott.ssa Erica Liberto (T)	erica.liberto@unito.it
Prof.ssa Patrizia Rubiolo (CD)	patrizia.rubiolo@unito.it
Prof.ssa Barbara Sgorbini (T)	barbara.sgorbini@unito.it

Advanced Pharmaceutical Nanotechnologies (APN)

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=z3lx

componente del gruppo afferente al Dottorato

nominativo	contatto
Prof.ssa Silvia Arpicco (CD)	silvia.arpicco@unito.it
Prof.ssa Roberta Cavalli (CD)	roberta.cavalli@unito.it
Dott.sa Barbara Stella (T)	barbara.stella@unito.it

Cardiovascular and Metabolic Pharmacology (CAMP)

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=9l4f

componente del gruppo afferente al Dottorato

nominativo	contatto
Prof. Massimo Collino (CD)	massimo.collino@unito.it

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Cellular Pharmacology (CP)

http://www.dstf.unito.it/do/gruppi.pl/Show?_id=f5dy

componente del gruppo afferente al Dottorato

nominativo	contatto
Dott.ssa Chiara Dianzani (T)	chiara.dianzani@unito.it
Prof. Roberto Fantozzi (CD)	roberto.fantozzi@unito.it

DIPARTIMENTO DI SCIENZE DELLA SANITA' PUBBLICA E PEDIATRICHE

www.dsspp.unito.it

Componenti del Collegio dei Docenti (CD) e Tutor (T) del Dottorato afferenti a questo Dipartimento appartengono al gruppo:

Environmental Hygiene

http://www.dsspp.unito.it/do/gruppi.pl/Show?_id=guh7

componenti del gruppo afferenti al Dottorato

nominativo	contatto
Prof. Roberto Bono (CD)	**roberto.bono@unito.it**
Prof.ssa Tiziana Schilirò (T)	**tiziana.schiliro@unito.it**

DIPARTIMENTO DI SCIENZE DELLA VITA E BIOLOGIA DEI SISTEMI

www.dbios.unito.it

Componenti del Collegio dei Docenti (CD) e Tutor (T) del Dottorato afferenti a questo Dipartimento appartengono ai gruppi:

Microbiology and Virology

http://www.dbios.unito.it/do/home.pl/View?doc=research/microbiology_and_virology.html

componente del gruppo afferente al Dottorato

nominativo	contatto
Prof. Giorgio Gribaudo (CD)	**giorgio.gribaudo@unito.it**

Plant Physiology

www.plantphysiology.unito.it

componenti del gruppo afferenti al Dottorato

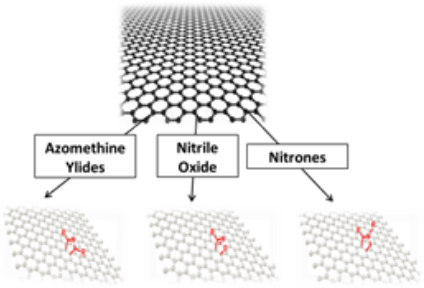
nominativo	contatto
Prof. Cinzia Bertea (T)	**cinzia.bertea@unito.it**
Prof. Massimo Maffei (CD)	**massimo.maffei@unito.it**
Dr. Andrea Occhipinti (T)	**andrea.occhipinti@unito.it**

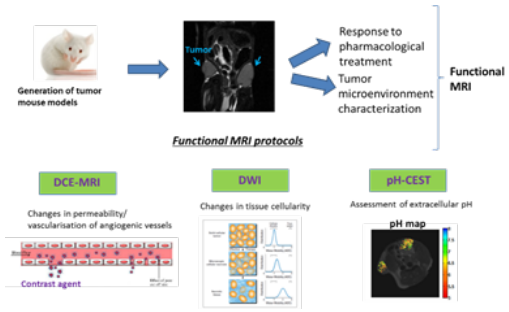
Structural and Functional Biochemistry

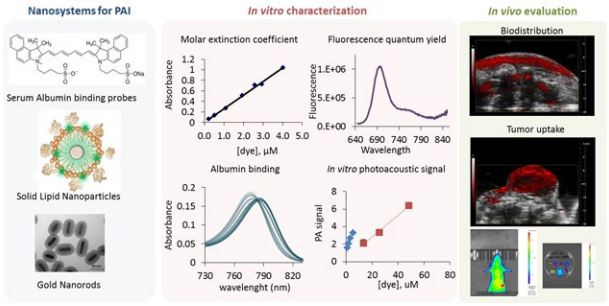
http://www.biochemistry-scienze.unito.it/Home.html

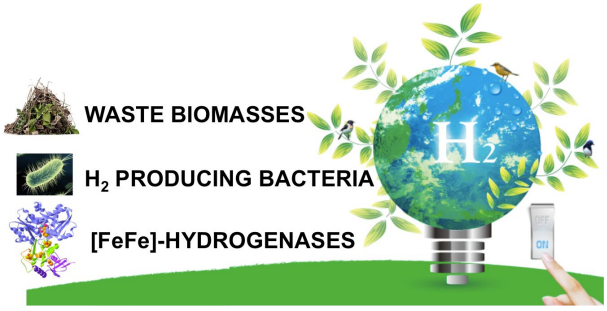
componenti del gruppo afferenti al Dottorato

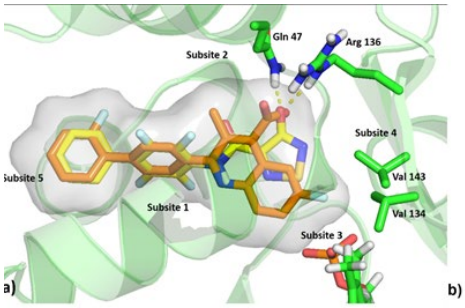
nominativo	contatto
Dott.ssa Giovanna Di Nardo (T)	giovanna.dinardo@unito.it
Prof. Gianfranco Gilardi (CD)	gianfranco.gilardi@unito.it
Prof.ssa Enrica Pessione (T)	enrica.pessione@unito.it
Prof.ssa Sheila Sadeghi (CD)	sheila.sadeghi@unito.it
Dott.ssa Francesca Valetti (T)	francesca.valetti@unito.it

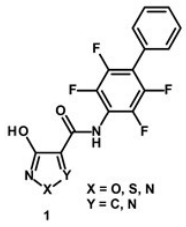


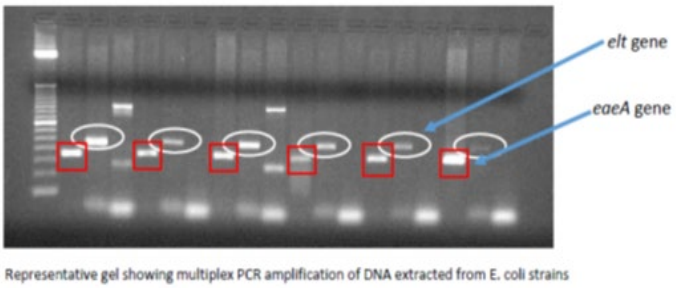




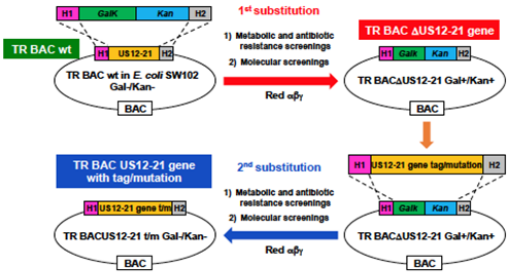








Representative gel showing multiplex PCR amplification of DNA extracted from E. coli strains



Manipulation of HCMV US12-21 gene family by BAC recombineering