



PhD Meeting 2019

September 18 - 20, 2019

University of Milano-Bicocca
Building U6, Room 26 1st Floor



MEETING ORGANISERS

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Program

Wednesday 18th of September 2019

09:00 – 09:30 Registration and Opening

Session I - Chemistry-thermodynamics of life origin – Part 1

/ PhD student's presentations

Chairs: Marco Orlando, Monica Oldani

09:30 – 10:20 *Invited lecture:*

T01. Luca De Gioia (University of Milano-Bicocca)

Thermodynamics and life (1):

From problematic coexistence to indissoluble relationship.

10:20 – 10:45 *PhD student's presentation: 3rd Year*

S01. Eleonora Torre (University of Milano-Bicocca)

Istaroxime improves diabetic diastolic dysfunction through SERCA stimulation

10:45 – 11:20 *Coffee break – Gallery at Ground Floor*

11:20 – 11:45 *PhD student's presentation: 2nd Year*

S02. Chiara Brambati (University of Milano-Bicocca – Diasorin spa)

DEVELOPMENT OF INNOVATIVE Q-LAMP ASSAY FOR DIRECT DETECTION AND AMPLIFICATION OF FLU A/B AND RSV GENOMES

11:45 – 12:10 *PhD student's presentation: 2nd Year*

S03. Stefano Bertacchi (University of Milano-Bicocca)

Enzymatic hydrolysis of Camelina meal for carotenoids production in oleaginous yeasts

12:10 – 12:35 *PhD student's presentation: 2nd Year*

S04. Alessandra Lodrini (University of Milano-Bicocca)

Cardiac Progenitor Cells-Derived Exosomes Protect Cardiomyocytes From Ageing-Induced Dysfunction

12:35 – 13:55 *Lunch – Gallery at Ground Floor*

Session II - Chemistry-thermodynamics of life origin – Part 2

/ PhD student's flash poster presentations

Chairs: Sara Marchetti, Simone Stucchi

14:00 – 14:50 *Invited lecture:*

T02. Luca De Gioia (University of Milano-Bicocca)

Thermodynamics and life (2):

A two-way ticket from Lost to Smart cities

14:50 – 15:10 *PhD student's flash poster presentation: 1st Year*

F01. Erika Casari (University of Milano-Bicocca)

Synthetic cytotoxicity to target DNA repair in cancer

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F02. Valentina Artusa (University of Milano-Bicocca)

Immunomodularity and anti-inflammatory activity of natural and synthetic molecules

F03. Gloria Campioni (University of Milano-Bicocca)

In vitro assessment of three dimensional models for the study of breast cancer metabolism

F04. Daniele D'Arrigo (University of Milano-Bicocca – Lugano Hospital)

Anti-inflammatory and regenerative potential of extracellular vesicles for osteochondral repair

15:10 – 16:10 *Coffee break with Poster Session – Gallery at Ground Floor*

Session III - PhD student's presentations

Chairs: Marco Orlando, Monica Oldani

16:10 – 16:35 *PhD student's presentation: 3rd Year*

S05. Luca Saponari (University of Milano-Bicocca – Magodoo Island)

Filling the gap: ecology of three major corallivores in the Republic of Maldives

16:35 – 17:00 *PhD student's presentation: 2nd Year*

S06. Alfredo Mento (University of Milano-Bicocca – Diasorin spa)

Alternative purification processes of C33 antigen for the detection of HCV infection

17:00 – 17.50 *Invited lecture:*

T03 Alex Graudenzi (Consiglio Nazionale delle Ricerche)

title to be announced

17:50 *End of Day 1*

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Thursday 19th of September 2019

Session IV - Neuro-Biomedicine – Part 1

/ PhD student's presentations

Chairs: Sara Marchetti, Simone Stucchi

- 09:30 – 09:55 *PhD student's presentation: 3rd Year*
S08. Monica Oldani (University of Milano-Bicocca)
Implementing the Cell Transformation Assay: mechanistic studies in cadmium-induced carcinogenesis
-
- 09:55 – 10:20 *PhD student's presentation: 3rd Year*
S09. Marco Orlando (University of Milano-Bicocca)
Antarctic glycoside hydrolase endolysins from a hitherto unexplored portion of sequence space
-
- 10:20 – 10:45 *PhD student's presentation: 2nd Year*
S10. Federica Bovio (University of Milano-Bicocca)
Cadmium effect on human superoxide dismutase 1
-
- 10:45 – 11:25 *Coffee break – Gallery at Ground Floor*
-
- 11:25 – 12:10 *Invited lecture:*
T04. Laura Russo (University of Milano-Bicocca)
Inducing Morphogenesis in Tissue Engineering: strategies, opportunities and state of art
-
- 12:30 – 13:55 *Lunch – Gallery at Ground Floor*

Session V - PhD student's presentations and flash poster presentations

Chairs: Eleonora Torre, Luca Saponari

- 14:00 – 14:25 *PhD student's presentation: 2nd Year*
S11. Antonio Marsella (University of Milano-Bicocca)
Rif2-mediated Regulation of MRX Activity at DNA Double-Strand Breaks
-
- 14:25 – 14:50 *PhD student's presentation: 2nd Year*
S12. Pooja Jayaprakash (University of Milano-Bicocca – University College Cork, UCC, Ireland)
Adaptive Laboratory Evolution to Enhance Organic Acid Tolerance in *Kluyveromyces marxianus* on Residual Biomass
-
- 14:50 – 15:15 *PhD student's presentation: 2nd Year*
S13. Luis Ferraz (University of Milano-Bicocca – University Minho, Portugal)
Membrane engineering to improve *Saccharomyces cerevisiae* robustness towards formic acid
-
- 15:15 – 15:35 *PhD student's flash poster presentation: 1st Year*
F05. Giulia Agostinetti (University of Milano-Bicocca)
DNA metabarcoding: improving biodiversity assessment through bioinformatics techniques
-
- F06. Nicola Tommasi** (University of Milano-Bicocca)
A molecular based assessment of plant pollinator interactions in sub Saharan agroecosystems: implications for sustainability
-
- F07. Alessandra De Giani** (University of Milano-Bicocca)
Impact of prebiotics and probiotics on gut microbiota and human health
-

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F08. Stefania Blasa (University of Milano-Bicocca)
Characterization of Biocompatible Substrate Effects on
Neuronal Circuit Constitution
TBA

15:35 – 16:40 *Coffee break with Poster Session – Gallery at Ground Floor*

Session VI - Neuro-Biomedicine – Part 2

Chairs: Sara Marchetti, Simone Stucchi

16:40 – 17:30 *Invited lecture:*

T05. Daniela Ferrari (University of Milano-Bicocca)
Human neural stem cells sources for cell therapies in the CNS
and a synopsis of the experience from phase I clinical trials

17.35

Group Foto

18:00

Aperitif at “SottoSopra” and End of Day 2

Friday 20th of September 2019

Session VII - PhD student's presentations

Chairs: Eleonora Torre, Luca Saponari

09:30 – 09:55 *PhD student's presentation: 3rd Year*

S14. Lorenzo Guzzetti (University of Milano-Bicocca)
Rediscovering Traditional Vegetables to Enhance Food and
Environmental Sustainability of Sub-Saharan Agriculture

09:55 – 10:20 *PhD student's presentation: 2nd Year*

S15. Jessica Frigerio (FEM2 Ambiente - University of
Milano-Bicocca)
Development of DNA barcoding-based technologies for the
authenticity, quality and safety assessment in food industry

10:20 – 11.00 *Coffee break – Gallery at Ground Floor*

11.00 – 11:25 *PhD student's presentation: 2nd Year*

S16. Sara Marchetti (University of Milano-Bicocca)
Epithelial-to-mesenchymal transition activation promoted by
Combustion-Derived Particles on alveolar epithelial cells

11:25 – 11:55 *PhD student's presentation: 3rd Year*

S17. Simone Stucchi (University of Milano-Bicocca)
Relationship between hyperglycemia and changes on
cartilage tissue associated with osteoarthritis and diabetes

12:30 – 13.30 *Lunch – Gallery at Ground Floor*

Session VIII - PhD student's flash poster presentations

Chairs: Eleonora Torre, Luca Saponari

13:30 – 13:55 *PhD student's presentation: 2nd Year*

S18. Fabio Rizza (University of Milano-Bicocca)
A computational approach to model disease-related systems
applications to neurofibromin and amyloid beta peptide

13:55 – 14:20 *PhD student's presentation: 2nd Year*

S19. Federico Fontana (University of Milano-Bicocca)

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	In-silico biomateriomics: coarse-grained molecular dynamics approach
14:20 – 14:45	<i>PhD student's flash poster presentation: 1st Year</i> F09. Fabrizio Beltrametti (Actygea srl) Multiple oxidative enzyme in ligninolytic fungi: chance or necessity? F10. Sofia Magli (University of Milano-Bicocca) New printable hybrid hydrogels for 3D cell model and tissue engineering applications F11. Riccardo Milanese (University of Milano-Bicocca) Glucose and Pyruvate Transport in Yeast: New Roles of Snf1/AMPK in the Control of Metabolism
14:45 – 15:45	<i>Coffee break with Poster Session – Gallery at Ground Floor</i>
Session IX - Round Table – PhD: the Day After	
Chair: Paola Esena	
15:45 – 17:15	<i>Invited speakers:</i> Vera Codazzi, Lorenzo Signori, Giulia Filippi, Ilaria Guerini title
17:15	<i>Poster award and concluding remarks - End of Day 3</i>

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**Thermodynamics and life (1): From problematic coexistence to
indissoluble relationship.**

Luca De Gioia

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

The relationships between the second principle of thermodynamics, which states that in an isolated system entropy cannot decrease, and life, which is necessarily associated to low entropy states, will be critically discussed, underlining also how this relationship evolved in the last 150 years.

Istaroxime improves diabetic diastolic dysfunction through SERCA stimulation

Eleonora Torre,¹ Alessandra Maria Lodrini,¹ Martina Arici,¹ Paolo Barassi,² Mara Ferrandi², Elisabetta Boz³, Claudio Bussadori³, Patrizia Ferrari², Giuseppe Bianchi², Marcella Rocchetti¹

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Calcium handling is generally impaired in heart failure (HF). Mechanisms that can restore cardiac relaxation improving cellular Ca²⁺ cycling, represent a promising therapeutic approach for HF. Istaroxime is a Na-K ATPase (NKA) inhibitor able to accelerate Ca²⁺ re-uptake into sarcoplasmic reticulum (SR) through SR Ca²⁺ pump (SERCA) stimulation by displacing the interaction between SERCA and its inhibitor, phospholamban (PLB).

The study aims to characterize Istaroxime effects in a model of mild diabetes (type 1) with diastolic dysfunction and preserved global systolic function. Istaroxime was tested at 100nM, mostly unaffacting NKA.

Streptozotocin (STZ)-treated rats were evaluated in vivo, in isolated left ventricular myocytes and in SERCA2a-enriched microsomes at 9 weeks after STZ injection in comparison to control (CTR) ones.

STZ-induced cardiomyopathy was characterized by impaired diastolic relaxation which was associated to reduced SERCA protein level and activity at the cellular level. In STZ group, action potentials (AP) were significantly prolonged at each cycle length; Ca²⁺ transients were characterized by slower decay, delayed onset and increased diastolic Ca²⁺. Istaroxime significantly stimulated SERCA activity and SR Ca²⁺ re-uptake after caffeine depletion in STZ group only. Moreover, Istaroxime reduced STZ-induced diastolic Ca²⁺ enhancement but not affected AP prolongation.

SERCA stimulation can be considered a promising therapeutic approach for diastolic dysfunction treatment.

Development of innovative Q-lamp assay for direct detection and amplification of flu A/B and RSV genomes

Chiara Brambati^{1,2}, Cinzia Pultrone¹, Pietro Vella¹, Marco Falcettoni¹, Chiara Montrasio¹, Luca Rubino¹, Chiara Lanceni¹, Sara Zonca¹, Elena Del Tordello¹, Marco E. Vanoni², Giulia Minnucci¹.

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Despite decades of surveillance and interventions, influenza viruses (especially types A and B strains¹) still represent a public health concern². Respiratory syncytial virus (RSV) also infects the respiratory system causing an influenza-like illness in infants, toddlers and high-risk adults³.

Despite accuracy of diagnosis based on clinical presentation, fast and specific tests are required to confirm virus-specific infection⁴.

PCR-based assays are commonly used⁵, requiring prior extraction of viral genomes to be amplified. This process is time consuming, needing skilled staff and equipped laboratories.

Loop Mediated Isothermal Amplification (LAMP) can solve several of these problems, as it is faster, precise, sensitive and specific.

The aim of this study is to develop a Q-LAMP-based assay, that, starting directly from patient's specimen, retro-transcribes, amplifies and differentially detects influenza A, B and RSV viruses in a single-step, optimized using reagents with improved sensitivity and specificity allowing clinicians to obtain results as early as possible.

The so-developed prototype Q-LAMP assay successfully amplifies influenza A H1N1, H3N2, influenza B plasmids and synRNAs, with similar efficiency and speed. The thermal protocol has been optimized introducing a sample-processing step ensuring detection of not-extracted viruses. Finally, reagents have been balanced and optimized allowing obtaining results in less than 60 minutes.

References

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4. Kumar, S. & Henrickson, K. J. (2012). *Clin. Microbiol. Rev.*, **25**, 344–361.
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Enzymatic hydrolysis of Camelina meal for carotenoids production in oleaginous yeasts

Stefano Bertacchi,¹ Nadia Maria Berterame,¹ Paola Branduardi.¹

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Biorefineries are key players in bioeconomy scenario, but their sustainability is strongly related to the feedstock origin. Therefore, biorefineries based on residual biomasses are increasingly of industrial interest, to overcome drawbacks of the use of edible resources. We focused our work on the exploitation of leftovers from *Camelina sativa* oil extraction; this biomass, called Camelina meal, is still rich in proteins and fibers, and, therefore, has applications in the feed industry^[1]. The project aims to exploit it for the production of carotenoids, high-value products used also in animal feed^[2], by fermentation of Camelina meal-derived sugars with *Rhodospiridium toruloides*, a natural producer of carotenoids^[3]. The biomass was hydrolyzed by enzymes, rather than acid, to release sugars, with a more sustainable approach. Different percentages of Camelina meal, together with different enzyme cocktails, concentrations and combinations, were tested, to optimize the saccharification step. Then, the obtained sugar mixture was converted by *R. toruloides* into the desired product. The process was developed either as a Separated Hydrolysis and Fermentation or as a Simultaneous Saccharification and Fermentation, and the data were compared. This work paves the way for obtaining a Camelina meal enriched in carotenoids by microbial cell factories-based processes.

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Cardiac Progenitor Cells-Derived Exosomes Protect Cardiomyocytes From Ageing-Induced Dysfunction

Alessandra Maria Lodrini¹, Edoardo Lazzarini², Sara Bolis³, Claudia Altomare³, Lucio Barile², Marcella Rocchetti¹

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Incidence of heart failure (HF) increases with age^[1], along with cardiomyocytes senescence^[2]. Current therapies for HF show limited efficacy^[3], hence innovative approaches are being investigated. Cardiac progenitor cells (CPCs) secrete nanoparticles (exosomes, EXO) enriched of cardioprotective factors^[4], which may exert an anti-senescent effect on cardiomyocytes. However, human models of *in vitro* cardiac ageing are missing. Thus, the aim is to derive a human model of cardiac ageing to assess EXO's capacity to ameliorate senescence-associated modifications.

Patient-derived CPCs were reprogrammed into hiPSCs and eventually differentiated into cardiomyocytes (hiPSC-CMs). Spontaneously beating hiPSC-CMs were exposed to doxorubicin (DOX) at sub-lethal concentration (0.2 μ M). DOX treatment induced senescence, as confirmed by activation of p21 and p16 pathways, increasing of SA- β -gal staining and hypertrophy in comparison to untreated cells (CTR). Biochemical and gene expression analysis revealed a switch from β -oxidative to glycolytic metabolism and subsequent decrease in ATP/AMP ratios. DOX-CMs also showed a prolonged QTc, effect reverted by therapeutic EXO treatment. Moreover, exposition to EXO prevented the insurgence of hypertrophy.

Our results suggest that our model recapitulates the phenotype of aged CMs, in terms of senescence markers and electrical and metabolic proprieties. Additionally, our findings indicate a role of EXO in reducing age-related modifications.

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Thermodynamics and life (2): A two-way ticket from Lost to Smart cities

Luca De Gioia

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

The most significant innovations discovered by life to exploit energy will be discussed using as background the principles of thermodynamics, with a focus on the three fundamental biological energy transduction mechanisms: substrate-level phosphorylation, electron transport-linked phosphorylation and electron bifurcation.

Synthetic cytotoxicity to target DNA repair in cancer

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Genomic instability is a fundamental aspect of human malignancies. Some of the genetic changes causing this instability render cancer cells vulnerable to DNA damage induced by conventional radio/chemotherapies. One problem of these therapies is the lack of selectivity for cancer cells resulting in side effects. To improve their selectivity, novel targetable proteins should be identified searching for genes that when mutated lead to synthetic cytotoxicity with cancer-related DNA repair defects. ATM is upregulated in some cancer cells ^[1] and its inhibition sensitize cancer cells to radiation ^[2]. For these reasons, ATM-inhibitors have been developed ^[3]. We plan to identify genetic profiles that could benefit from the use of ATM inhibitors by identifying tumor-specific mutations that are synthetically lethal with an ATM inhibitor in the presence of DNA damaging agents. Given the conservation of DNA repair pathways, we are using the yeast *S. cerevisiae* for searching extragenic mutations that are synthetically lethal with the *TEL1* deletion in the presence of camptothecin, a topoisomerase I inhibitor used in cancer therapies ^[4]. We have identified a missense mutation in the *DPB4* gene, encoding a protein that belongs to both DNA pol epsilon complex and the chromatin accessibility complex Isw2. We are characterizing such mutation.

References

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Immunomodularity and anti-inflammatory activity of natural and synthetic molecules

Valentina Artusa¹, Chiara Arduini², Fabio Alessandro Facchini¹, Andrea Luraghi¹, Jessica Negrini¹, Carlotta Ciaramelli¹, Alessandro Palmioli¹, Cristina Airoidi¹, Carlo Rossetti², Francesco Peri¹

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The innate immune response is a universal mechanism of host defence against infection^[1]. Toll-like receptors (TLRs), play an essential part in the perception of microbes and shape the complex host responses^[2]. Among the pharmaceutically relevant concepts, Toll-Like Receptor 4 (TLR4), is one of the most promising^[3]. TLR4 signal pathway plays an important role in initiating the innate immune response and its activation by bacterial endotoxin is responsible for chronic and acute inflammatory disorders^[4]. TLR activation leads to the production of proinflammatory mediators and thus TLR signaling must be properly regulated. Furthermore, TLR ligand-induced microRNAs, including miR-146a and miR-155, are involved in regulating inflammatory mediators^[5]. The aim of this project is to understand the underlying mechanisms of TLR4 inhibition (antagonism) or activation (agonism) by small organic molecules of both natural and synthetic origin. On one hand, findings about the TLR4 modulation activity of molecules derived from plant extracts could help stimulate additional structure-based drug design efforts in these inflammation and immune response areas. On the other hand, changes in miRNA expression by newly synthesized TLR4 ligand stimulation could be interesting from the viewpoint of vaccine adjuvants. Preliminary results of *in vitro* studies using THP-1-derived macrophages showed an interesting capability of a MPLA-like synthetic molecule, FP18, to stimulate the production of pro-inflammatory cytokines (TNF-alpha and IL-1beta) in a dose-dependent manner and to induce the expression of miR-146a and miR-155 in an LPS-like fashion. In parallel, different fractions derived from coffee extracts, tested for their putative anti-inflammatory activity, have shown the ability of inhibit the production of LPS-induced TNF-alpha, while the reduction of LPS-induced IL-1beta production was not significant. However, further studies are required to identify the molecular variants responsible for these biological activities and their mechanisms of action.

References

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***In vitro* assessment of three dimensional models for the study of breast cancer metabolism**

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The worldwide leading cause of cancer-related death in women is breast carcinoma (15% of cancer deceases in females)^[1]. Endocrine therapy, chemotherapy and strategies targeting the primary tumor have markedly improved survival, but metastasis formation, development of drug resistance and cancer relapse remain the major underlying cause of breast cancer mortality^[2]. Altered metabolism has been recognized as one of the major mechanisms underlying therapeutic resistance^[3], tumor progression and spread throughout the organism^[4]. Cancer metabolism can influence and shape tumor microenvironment and vice-versa^[5], thus it is important to develop *in vitro* models that can simulate tumor architectural complexity. For this purpose, we are developing three-dimensional models of breast carcinoma, starting from established cell lines (triple negative breast cancer: SUM159PT, MDAMB231; hormone receptors positive breast cancer: MCF7) or Patient Derived Xenografts^[6]. We first concentrated on nutritional and pharmacological modulation of the ability of the cell lines to form spheroids, characterized through the expression of (hormone) differentiation and positional markers. We use the Seahorse analyser and specific mitochondrial fluorescent probes for quantitative determination of mitochondrial and glycolytic activity. We plan to collect metabolomic and transcriptomic data to constrain computational models^[7] able to identify potential metabolic targets and predict response to personalised therapies.

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Anti-inflammatory and regenerative potential of extracellular vesicles for osteochondral repair

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Osteoarthritis (OA) is a multifactorial degenerative disease with an increasing incidence on the ageing population. The major issues consist in the lack of both early biomarkers and effective therapeutic treatments^[1], as well as methods through which it is possible to evaluate and compare innovative approaches. In this scenario, we first developed and optimized a microfluidic model resembling an arthritic joint which allows the evaluation and the comparison of new biological treatments for OA, like stem cell therapy (with mesenchymal stem cells) and extracellular vesicles (EVs).

EVs represent one of the most promising approach not only in the research of early and stage-specific OA markers but also in the development of new and effective regenerative clinical treatments^[2]. For this reason, we will evaluate the potential role of EVs both in the therapeutic and in the diagnostic fields. In particular, within the 3D environments of the device previously developed we will assess and compare the immunomodulatory and regenerative capabilities of EVs secreted by various MSCs types. Regarding the diagnostic field, the entire EVs spectrum of joints with distinct load-bearing in various pathophysiological conditions will be characterized and compared, giving indications on the possible use of EVs as a diagnostic tool.

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Filling the gap: ecology of three major corallivores in the Republic of Maldives

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Coral reefs are among the most diverse marine ecosystem on Earth, although, several natural and anthropogenic threats are increasing its degradation. Despite being worldwide studied, there is a general lack of information on the role of coral predators in reef degradation, especially in remote locations. In this context, we aimed at filling this gap by an extended monitoring program of the major corallivores, the seastars *Acanthaster planci* and *Culcita* spp., and the snail *Drupella* spp., in the Republic of Maldives. The target organisms were monitored separately on 37 locations, in two Atolls from 2015 to 2019. The surveys allowed to describe distribution, outbreak densities, population structure and feeding preferences of the three corallivores for the first time in the central Maldives. Occurrence was mostly homogeneous and population structure was mainly constituted by adults. Only *A. planci* showed outbreak, excluding 1 occasion for *Culcita* spp.. Predation pressure focused on few genera, *Pocillopora*, *Acropora* and *Porites*, and specific colony size with only *Culcita* spp. focusing on recruits < 10 cm. Besides giving an ecological baseline, such results highlighted the potential for local shift in coral community. In addition, we discussed the ecology of the target corallivores related to the current environmental changes.

Alternative purification processes of C33 antigen for the detection of HCV infection

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Hepatitis C virus (HCV) causes chronic liver injury in most instances, cirrhosis and hepatocellular carcinoma, so it's very important to have an early, specific and sensitive detection of HCV infection^[1]. The most widely used test for HCV diagnosis is the measurement of anti-HCV antibody in serum by using chemiluminescent immunoassay or enzyme immunoassay method^[2]. Many HCV-antigens, such as the core antigen NS3 and NS4, are usually used in the diagnostic kit for HCV since they lead the anti-HCV antibodies production^[3]. The current DiaSorin LIAISON® HCV IgG assay relies on these antigens, in which the core and NS4 antigens are immobilized on solid phase and NS3 is biotinylated and lyophilized.

One of the main antigens used for the HCV diagnosis is C33 protein, which represents a large portion of helicase domain of NS3 and it consists of immunodominant epitopes on the nucleocapsid region of HCV genome^[4].

During my second year of Phd we worked on the improvement of alternative purification processes of C33 antigen exploiting the intein auto-cleavage activity and the Elastin-like polypeptides reversible aggregation, at the same time we explored an innovative method for the site-specific biotinylation of C33 antigen that uses the Split-intein system^[5].

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Implementing the Cell Transformation Assay: mechanistic studies in cadmium-induced carcinogenesis

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Carcinogenesis is one of the areas of major concern in the context of 3Rs and alternative approaches to the mouse bioassay are needed. Among these, the Cell Transformation Assays (CTAs) are one of the *in vitro* models for the identification of potential human carcinogens^{1,2}, especially in the context of an integrated approach to testing and assessment (IATA)³. These assays, limited to the screening of compounds, actually, are employed for studying the process of transformation. In this context, we exploited the use of CTAs for mechanistic studies of cadmium-induced carcinogenesis. We carried out a whole-genome analysis to evidence deregulated pathways in C3H10T1/2Cl8 after 24h of cadmium treatment or in foci-derived transformed cells. Consequently, according to *in silico* analyses, we focused on metabolic rewiring and mitochondrial structure and function. In more details, we applied seahorse methods, spectrophotometric enzymatic assays, laser scanning confocal fluorescence microscopy and flow cytometry technique. The essential aspect of this approach was considering many variables at once⁴ by integrating bioinformatics tools combined with laboratory work. We are confident that the joint use of many techniques could develop a mechanistic-based method for improving the reliability of CTAs, leading to the Reduction of animal used.

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Antarctic glycoside hydrolase endolysins from a hitherto unexplored portion of sequence space

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Glycoside Hydrolases 19 (GH19) are enzymes active in the degradation of β (1,4) glycosidic bonds between N-acetyl glucosamine units. They have been described in plants¹, Eubacteria², a fungal³ species, and recently also as phage-related endolysins⁴.

Two GH19 coding genes (lys177 and lys188) were identified in the genome of *Pseudomonas* Ef1, a bacterial symbiont of the Antarctic ciliate *Euplotes focardii*. The expressed products, LYS177 and LYS188, were produced in *E. coli* cells and purified.

They proved to be positive to a standard assay of lysozyme activity, with an optimal temperature below 35°C, thermolability at higher temperatures and a relatively high activity at 5°C. This is in accordance with the close phylogenetic relations of these enzymes with other GH19 endolysins.

Endolysins are attracting interest as potential antimicrobial agents, particularly for the emerging bacteria resistance against classical antibiotics. In order to conclude this study the GH19 sequence space was mined to address the relationship between chitinolytic and lysozyme activities and provide a database for new potential sources of antimicrobials.

Moreover, LYS177 activity against Gram – bacteria and its relatively fast inactivation during incubations even at room temperature makes it a good candidate for further studies in food/beverage low-temperature preservation.

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Cadmium effect on human superoxide dismutase 1

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Human superoxide dismutase 1 (SOD1) is a 32 kDa protein responsible for superoxide anions dismutation. It is a homodimeric metalloenzyme with each monomer binding one copper and zinc ions. Zinc plays roles in stabilizing SOD1 structure, while the copper ion is responsible for the catalytic activity. Mutated SOD1 in amyotrophic lateral sclerosis is implicated in the formation of toxic aggregates¹; recent studies suggested that SOD1 demetallation or aberrant metallation could be key factors for aggregation.²

Cadmium (Cd) is a widespread toxic environmental contaminant, due to anthropogenic activities. It interferes with essential metal ions homeostasis, substituting zinc, copper and iron in proteins and affecting their structures and functions.

In this study the effect of different mixtures of Cu, Zn and Cd on recombinant GST-SOD1, expressed in *E. coli* BL21, was investigated. After setting the optimal Cu and Zn concentrations for SOD1 activity, enzyme activity and expression were evaluated in the presence of different Cd concentration. Cd causes a dose-dependent reduction in SOD1 activity, while the expression remains constant. Similar results are obtained on SOD1 from human SHSY-5Y cells treated with Cd for 24h and 48h: the enzymatic activity decreases in a dose- and time-dependent way, while the protein expression is constant.

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**Inducing Morphogenesis in Tissue Engineering: strategies,
opportunities and state of art**

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The development of strategies to induce tissue and organ morphogenesis is of great interest for basic and translational research. The signals driving cells to differentiate and therefore generate different organs must be understood and ideally must be exploited in regenerative medicine. The development of in vitro models mimicking different cell microenvironments will be also useful to clarify and overcome many unresolved pathologies that affect the population (i.e., cancer, chronic diseases). The way to induce cell differentiation and tissue regeneration involves both the biochemical and physical signals of cell microenvironment. In particular, the extracellular matrix components that surround cells and their structural organization are currently under investigation to define the role of different actors in tissue regeneration. Here in this lecture, the current strategies and the unresolved challenges of tissue engineering will be presented.

Rif2-mediated Regulation of MRX Activity at DNA Double-Strand Breaks

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DNA double-strand breaks (DSBs) are among the most cytotoxic DNA lesions, because failure to repair them can lead to genome instability. DSBs can be repaired by either nonhomologous end joining (NHEJ) ^[1], or homologous recombination (HR) ^[2]. The key process in determining which pathway is used to repair DSBs is the initial processing of the DSB ends. The yeast Mre11-Rad50-Xrs2 complex (MRX) has structural and enzymatic activities to initiate DSB resection and to maintain the DSB ends tethered to each other for their repair. Several studies have shown that ATP binding and hydrolysis activities of the Rad50 subunit regulate DNA binding, tethering and nuclease functions of the MRX complex.

In budding yeast, MRX is known to interact with Rif2, which negatively regulates telomerase-mediated telomere elongation. We have previously shown that Rif2 enhances the ATP hydrolysis activity of Rad50 and attenuates MRX function in end-tethering. This observation, together with the finding that the lack of Rif2 by itself increases both end-tethering and NHEJ, suggests that Rif2 can regulate MRX activity at DSBs by modulating ATP-dependent conformational changes of Rad50 ^[3]. To better understand the crosstalk between Rad50 and Rif2, we have searched for *rad50* mutants that phenocopy *RIF2* deletion and therefore that increase both NHEJ and end-tethering. We identified a mutation in Rad50 that is located on the surface of the protein, suggesting that it can affect a possible Rif2-Rad50 interaction. We will present data regarding the structural and functional characterization of this Rad50 mutant variant.

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Adaptive Laboratory Evolution to Enhance Organic Acid Tolerance in *Kluyveromyces marxianus* on Residual Biomass

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In biorefineries the biochemical conversion of lignocellulosic feedstocks to advanced biofuels and other commodities requires the development and usage of efficient, robust, versatile microbial cell factories with innate or engineered traits. Regrettably, the pretreatment of lignocellulosic biomass, necessary to make the sugars accessible, releases organic acids that are inhibitory to the production microorganisms^[1]. *Kluyveromyces marxianus*, a non- *Saccharomyces* yeast is not an exception despite several advantages that it possesses, such as unique ability to grow at high temperatures (up to 45°C) and to utilize broad range of substrates^[2], including the C5 sugars that are present in lignocellulosic biomass. Here, we use an Adaptive Laboratory Evolution (ALE) strategy to enhance the organic acid tolerance of *K. marxianus* on sugar beet pulp, a residual lignocellulosic biomass composed of C5 and C6 sugars along with acetic and lactic acid that inhibit growth at low pH. ALE was performed by sequential serial passages in shake flasks and the performances of wild type strain and evolved isolates were tested by pH-gradient acetic acid plate assays and growth kinetics in liquid media. By comparing the specific growth rate and the duration of the lag phase, *K. marxianus* variants with improved tolerance were selected.

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Membrane engineering to improve *Saccharomyces cerevisiae* robustness towards formic acid

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Utilization of lignocellulosic-biomasses for bio-based microbial processes requires a pretreatment step to release fermentable sugars. Simultaneously, microbial inhibitors, such as weak organic acids (WOAs) (for example formic acid) are released. WOAs enter in the cell by simple diffusion, especially at low pH, and once inside, they dissociate into proton and anion, their accumulation can cause growth arrest¹. Furthermore, WOAs, such as lactic acid, can be produced by microorganisms to be used in several industries. Plasma membrane plays a pivotal role controlling the inward/outward flux of WOAs². *Saccharomyces cerevisiae* is widely used as a cell factory in bio-based processes, here we evaluated the capacity of a wild-type strain and a strain engineered for lactic acid production to cope with formic acid stress alone or in combination with the lactic acid produced. We evoked a membrane rewiring using a global transcription machinery engineering approach, focusing on the modulation of the transcription factor *ECM22*, involved in the regulation of ergosterol biosynthesis. *ECM22* deletion has a positive impact on yeast robustness towards formic acid. We will illustrate how the increased robustness correlates with a rearrangement of the plasma membrane composition and how this can impact lactic acid production.

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DNA metabarcoding: improving biodiversity assessment through bioinformatics techniques

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High Throughput Sequencing (HTS) sequencing is now a routine technique applied in several biology fields^[1]. Combined with molecular taxonomy (DNA barcoding) principles, the resulting data allow to explore biodiversity based on DNA traces, enabling researchers to perform surveys on any kind of matrix, including high degraded DNA matrices as water, feces or food (DNA metabarcoding)^[2]. The production of huge amounts of data makes the bioinformatic analysis mandatory: briefly, the reconstruction of the taxonomy from DNA fragments is defined by computational steps that are peculiar for each marker gene and substantially they could be divided in data cleaning, taxonomy assignment of amplicons and data analysis. Any step of the analysis could affect the quality of the result, introducing biases in species detection lead to incorrect interpretation of the data. Here, we present few case studies of DNA metabarcoding analysis, ranging from the survey of different environmental matrices up to the characterization of composition of processed food products^[3]. These cases demonstrate the versatility of the approach and its applicability, but also they show some concrete pitfalls that need to be overcome to improve the whole approach and increase its reliability.

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A molecular based assessment of plant pollinator interactions in sub Saharan agroecosystems: implications for sustainability

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The global decline affecting pollinator insects all over the world is increasing the scientific efforts in understanding more about pollination ecology. Despite its importance, only a few studies are available about pollination issues from developing countries, including Sub Saharan Africa. The analysis of pollinator-plant interactions and their changes in relation to anthropogenic stressors and landscape management allows to better plan effective conservation efforts. Here, we analysed pollination networks using an ITS2 DNA metabarcoding approach to reveal the taxonomic composition of pollen carried by insects to understand interactions with flowers, coupled with COI DNA barcoding to identify the pollinator species in Tanzania, Arusha region. During the Tanzanian dry season and short rain season we sampled 285 wild pollinators, observed foraging actively on flowers, in 28 localities belonging to three main agricultural landscapes: organic, intensive and urban. We successfully characterized pollen of at least 157 plant species from more than 18 pollinator species with interesting conservation and sustainability outcomes. For the very first time this integrated approach based on field ecology, bioinformatics and ecological modelling allowed to shed light on plant-pollinators interactions from Sub Saharan Africa with new insights for landscape and biodiversity management policies.

Impact of prebiotics and probiotics on gut microbiota and human health

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The human gut microbiota is a complex ecosystem and several disorders were associated with dysbiosis. Therefore, approaches trying to restore or prevent gut imbalances represent a new strategy to improve human health. In this context, the first year of my PhD project focused on the evaluation of the probiotic properties of specific *Lactobacillus* and *Bifidobacterium* strains and on the assessment of their possible combination with carbohydrate prebiotics in order to support their growth and efficacy. Among the previously characterized probiotics ¹⁻², *Lactobacillus plantarum* LP, *Lactobacillus rhamnosus* LRh and *Bifidobacterium animalis* subsp. *lactis* BL showed good probiotic features, specifically they had the strongest antimicrobial activity against several pathogens. Furthermore, LP could secrete metabolites such as bacteriocin-like compounds³. In order to evaluate the best prebiotic for a suitable combination, *in vitro* fermentations revealed that mixtures of fructooligosaccharides of different degree of polymerization were highly fermented by most of the probiotic strains, while limited growth was observed on inulin with a degree of polymerization up to 60⁴. These results suggest that the strains showed a good probiotic potential and the ability to grow on different carbohydrates, supporting the possibility of a pre/probiotics combination for further studies, including *in vivo* trials.

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Characterization of Biocompatible Substrate Effects on Neuronal Circuit Constitution

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Neuronal cell loss, due to neurological injuries or neurodegenerative diseases, represents one of the most important problems in public health. The neuronal tissue has a limited regenerative ability and needs supportive technologies for its repair^[1]. Several studies have been performed on cell differentiation ability when subject to mild heat^[2]. In this project we are studying two methods to promote the differentiation of neuroblastoma F-11 cell line in serum-deprived medium: bulk heating and photothermal stimulation by near infrared radiation (NIR) of Prussian Blue^[3] and Gold Nanostar^[4] nanoparticles in biocompatible substrates, at 41.5°C, 39.5°C and 37°C. The preliminary results obtained by measuring cell growth, neurite elongation and the number of dendrites per neuron suggested nanoparticle ability to promote differentiation, with a statistically significant difference in neurite elongation at 41.5°C versus 37°C (p=0.004). Functional analysis of neuronal circuits using the Patch-Clamp technique were also promising, showing that irradiated F-11 cells reached a higher differentiation compared to control cells. The results we obtained for cell differentiation *in vitro* by the combination of irradiation, nanoparticles and biocompatible materials suggest that this approach could be applied in the near future *in vivo* as a new strategy to treat neurodegenerative disorders and peripheral nerve regeneration.

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Human neural stem cells sources for cell therapies in the CNS and a synopsis of the experience from phase I clinical trials

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Cell based approaches remain one of the most promising areas of investigation for the development of effective experimental therapies for central nervous system disorders at large. A wide variety of cell donor sources, spanning from cells from the surrenal gland to primary fetal brain cells have been now tested in clinical settings. Thus, a lot of emphasis goes on the source of donor cells to be used under different therapeutic settings, for many of the cell systems used so fare pose serious procurement, technical or ethical limitations or concerns. Appropriate answers to this situation may be found with stem cells. The inherent functional plasticity of stem cells allows them to carry out a plethora of potential therapeutic actions, spanning the replacement of dead cells, immunomodulation, antiinflammatory, trophic, homeostatic, scavenging and toxicity blunting effects. I will describe our current Good Manufacturing Practice (cGMP) protocol and experience with human fetal brain stem cells (hNSCs) for the establishment of continuous, stable, plentiful and standardized cell lines that are amenable for certification under clinical good manufacturing practice standards (European Medicine Agencies). We report how such cells successfully obtained cGMP certification (aM 154/2018) and were used in a Phase I clinical trial (EudraCT 200901448439) in which 18 ALS patients received multiple grafts of these cells. The trial was completed successfully, follow up exceeding three years landmark. Safety and efficacy data will be discussed, followed by presentation of an ongoing phase I clinical trial with intracerebroventricular injection of the same hNSCs to treat pernicious, secondary progressive multiple sclerosis patients (EudraCt 201500485537).

Rediscovering Traditional Vegetables to Enhance Food and Environmental Sustainability of Sub-Saharan Agriculture

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Among the huge African plant biodiversity, many traditional vegetables (TVs) are underutilized in Sub-Saharan agriculture due to the spread of staple crops highly water-demanding and usually requiring tillage and agrochemicals to grow. Furthermore, they show an unbalanced equilibrium between macro and micronutrients, thus impoverishing people diet¹.

This year work focused on the evaluation of growth, yield and nutritional parameters of two African TVs (*Vigna unguiculata* L. Walp. and *Corchorus olitorius* L.) grown under conservation agriculture (CA) management (no tillage and cover crops maintenance) and low irrigation regime, typical agronomic conditions of many sub-Saharan farms.

Results showed that these TVs are able to grow and yield comparably among treatments, despite a decrease of total foliage produced without irrigation.

Furthermore, *V. unguiculata* seeds showed a relevant amount of aminoacids (4-33.67 mg*g⁻¹) and the occurrence of peptides able to kill selectively E705 tumor colon cells, but not healthy lines. *C. olitorius* leaves revealed huge amounts of folates (up to 30 µg*g⁻¹).

Notably, these phytochemicals were not influenced by treatments, thus confirming the capability of such species of providing healthy diet also if cultivated under CA management. Therefore, these TVs are good candidates to promote and couple food and environmental sustainability of Sub-Saharan agroecosystems.

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Development of DNA barcoding-based technologies for the authenticity, quality and safety assessment in food industry

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In the agri-food sector, quality is increasingly demanded by consumers. Chemical indicators are currently used, but these frequently do not allow to detect some contaminants and counterfeits. A suitable bio-molecular identification and traceability system could be used to assess the quality of raw materials up to the finished food products. DNA barcoding is a universal tool to identify species, and it currently allows for routinely market analysis ^[1]. However, DNA barcoding technique has some limits. Industrial treatments could alter DNA quality of raw material, therefore this analysis could be challenging to apply. Furthermore, this technique sometimes fails in distinguishing close related species due to their poor nucleotide sequences variability at standard loci (up to 1300 bp). Finally, it can't be applied for multispecies products. In this study we investigated the efficacy of DNA barcoding, its modification and others DNA-based tools to define a suitable industrial traceability system to improve quality and safety. Specifically, we tested minibarcoding regions (<200 bp) to identify highly processed products like food supplements ^[2]. We also set up a species-specific tool to distinguish close related contaminants of flours such as *Brassica nigra* and *Sinapis alba* ^[3]. Finally, we tested metabarcoding analysis for the quality assessment of novel food insect based, analysing both plant and bacteria DNA profile.

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Epithelial-to-mesenchymal transition activation promoted by Combustion-Derived Particles on alveolar epithelial cells

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Combustion-derived particles (CDPs) are linked to several respiratory diseases, including lung cancer^[1]. Epithelial-to-mesenchymal transition (EMT) is a crucial step in lung cancer progression, involving morphological and phenotypical changes^[2]. This study aims at comparatively investigate how exposure to CDPs from different biomass sources promotes EMT on human A549 lung cells. CDPs (PM10) were collected from a stove fuelled with pellet, charcoal or wood respectively. A time course and dose response evaluation on cell viability and pro-inflammatory response was performed to select the best conditions for EMT-related studies. A549 increased motility and invasiveness were investigated after 72 h of exposure to 2.5 µg/cm² CDPs. EMT-associated protein expression was also investigated.

Long-term exposure revealed that CDPs affect cell viability and inflammatory response differentially. Moreover, they promote EMT activation, inducing alterations in cell migration ability and invasion capacity. Process activation involves also loss of cell-to-cell contacts and gaining of mesenchymal cell markers.

In conclusion, pro-carcinogenic effects induced by CDPs on epithelial cells can be multiple and diverse according to the chemical composition and properties of particles. Thus, this research highlights the importance of studying CDPs from different biomass sources and targeting those airborne particles that could represent a serious risk for lung cancer development.

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Relationship between hyperglycemia and changes on cartilage tissue associated with osteoarthritis and diabetes

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Osteoarthritis (OA) is the most common form of degenerative joint disease and is often associated with diabetes, obesity and ageing¹. OA is characterized by focal degradation of articular cartilage due to changes in the behaviour of the chondrocytes^{1,2}. Experimental findings support the hypothesis that diabetes is an independent risk factor for OA¹. In the first part of this research project we have seen what is the effect of high glucose environments on chondrocyte cell line C28/I2. Therefore, we have cultured cells using different glucose concentration and we have analysed the cell growth, the expression of cytoskeletal proteins, ROS levels and apoptosis. Our results indicate that at high glucose concentration chondrocytes grow slowly, exhibit cytoskeletal alteration and increasing ROS levels. In addition, chronic hyperglycaemia may induce glycation of some cartilage matrix proteins, such as collagene³⁻⁵. Therefore we have investigated of glycation on chondrocyte behaviour, using glycated collagen films on which cells show minor growth rate, increased MMP13 production and apoptosis. Furthermore, a new 3D gelatin-base sponge helpful to do a cell based cartilage repair was developed. Physical and chemical properties of these new gelatin-based sponge were evaluated and the new sponge was used for 3D chondrocyte culture. In the third part of this project we evaluated the role of Prdx6 in primary chondrocytes after FN-f, H2O2, Menadione and DMNQ treatment. Prdx6 expressions in chondrocytes is not influenced by ages. Moreover, the effect of Prdx6 overexpression on chondrocytes MAPK signalling treated with Men, IGF-1 and FN-f was evaluated.

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A computational approach to model disease related systems applications to neurofibromin and amyloid beta peptide

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Neurofibromin is a multidomain protein acting as a ras-gap, mainly in the nervous system. Mutations in its gene give rise to Neurofibromatosis type 1, an autosomal dominant genetic disease that lead affected patients to develop, among other symptoms, cancers in the peripheral nervous system^[1]. Molecular dynamics simulations were used to investigate the structural effects of mutations in the Sec14-Ph domain of neurofibromin, whose physiological function has not been fully understood yet^[2]. Further we studied the binding properties to ras of two physiological isoforms of neurofibromin that have been shown to differ in the gap activity^[3].

Amyloid beta (A β) is the main component of amyloid plaques founded in Alzheimer's patients^[4]. Still far from a comprehensive understanding of the origins of this disease, A β -mediated oxidative stress has been suggested to play a major role in Alzheimer's related brain damages^[5]. A combination of molecular dynamics and quantum chemistry calculations (DFT) have been exploited to elucidate the possible paths of OH radical propagation within A β ₁₋₁₆ peptide in complex with Cu(II) in different copper coordination models suggested by previous DFT investigations^[6]. We observed that the most likely coordination models active in the OH propagation to 2-oxo histidine are those with two histidine ligands in line with the experiments.

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***In-silico* biomateriomics: coarse-grained molecular dynamics approach**

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Biomateriomics aims to elucidate the structure-properties relationship in nature-inspired materials across different scales, from nano- to macro-. Thanks to the recent progresses in the field of computational chemistry, it is possible to investigate several features of biomaterials.^{[1][2]} MARTINI coarse-grained molecular dynamics (CG-MD) simulations have proven to be suitable for the elucidation of self-assembling peptide (SAP) biomaterials organization.^{[3][4]} Indeed, MARTINI CG-MD simulations, combined with dedicated software such as home-made Morphoscanner, allow to investigate conformational aggregation patterns of SAPs.^{[5][6]} Steered MARTINI CG-MD simulations found limited applications in the investigation of mechanical properties of proteinaceous material, due to the lack of details about secondary structure transitions.^[7] In order to overcome this limitation, GoMARTINI approach has been optimized and adopted to investigate SAPs fibril mechanical features.^[8] This innovative approach has been used to characterize the failure mechanisms of different SAPs fibrils, shedding a new light on the effect of functionalization on nano-mechanical SAPs features.^[9] The combination of the abovementioned approaches, will allow to develop new coarse-grained models (at higher scale) of SAPs, in order to shorten the gap between empirical mechanical testing and theoretical simulations, and, as a consequence, new classes of functionalized and bio-conjugated SAPs, with tuned functionalizations and mechanical features.

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Multiple oxidative enzyme in ligninolytic fungi: chance or necessity?

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White rot and brown rot ligninolytic fungi are known for being widespread, adaptable to different environmental conditions and for being producers of a large number of oxidative enzymes (mainly laccases and peroxidases) of industrial interest ^[1, 2].

It has been largely demonstrated that, several oxidative enzyme-isoforms or closely related enzymes with apparently the same substrates or function are produced by the same fungal isolate ^[2]. Although the production of laccases and peroxidases has been largely investigated in academic and industrial settings, limited knowledge is available on the ecological meaning of the production of the different oxidative enzymes-isoforms by a single species or subspecies .

By use of solid-state fermentation, we have evidenced that *Coriolopsis gallica* MUT 3379 is able to produce different enzymes/isoforms with oxidative activity when grown on milled wood from different plant species. We therefore suggest that this white rot fungus is able to modulate the set of its oxidative enzymes based on the specific wood type colonized. In view of an industrial production, the use of the correct growth substrate is the starting point for the massive production of different oxidative enzyme-isoforms.

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New printable hybrid hydrogels for 3D cell model and tissue engineering applications

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The 3D bioprinting method represents a transformative approach that revolutionized medical and pharmaceutical research areas^[1]. 3D bioprinting is becoming an increasingly common technique due to the potential to provide personalized medicine approaches, structural complexity and on-demand fabrication. This technology has various applications, as regenerative medicine, one of the greatest interdisciplinary challenges, aiming to trigger the biological regeneration of failed human tissues and organs^[2]. In addition, 3D cell-printed structures have been used as 3D functional models for drug screening and cancer research. Indeed, it is increasingly evident that 3D cell culture are better models than the traditional 2D monolayer culture due to improved cell–cell interactions, cell–ECM (extracellular matrix) interactions, and cell populations and structures that resemble *in vivo* architecture^[3]. In this contest, hydrogel-based biomaterials are considered of wide interest because they have several features suitable to mimic extracellular environment^[4]. Aim of the work is the development of new 3D printable hydrogel model for cell cultures and tissue engineering. Proteinaceous and polysaccharidic polymers have been functionalized to produced hydrogels with tunable stiffness. The produced hydrogels have been used for cell encapsulation and 3D bioprinting procedure.

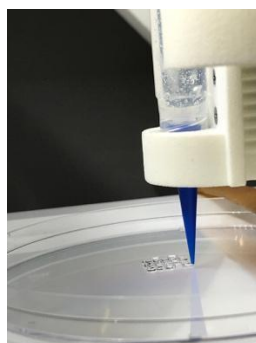


Fig. 1 3D printing test of thiolated hyaluronic acid.

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Glucose and Pyruvate Transport in Yeast: New Roles of Snf1/AMPK in the Control of Metabolism

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Metabolic transporters play a pivotal role in the regulation of metabolism, despite this their interplay with signal transduction pathway is still poorly understood.

We recently described an interaction between the Snf1/AMPK protein kinase and the pyruvate metabolism ^[1]. *SNF1* deletion have been shown to rewire yeast metabolism under glucose repression, increasing pyruvate transport into mitochondria and respiration ^[1]. Pyruvate import into mitochondria is mediated by MPC complex, composed by the constant subunit Mpc1, and one of the alternative subunits Mpc2 or Mpc3 ^[2]. Since the lack of knowledge about these transporters, we are investigating the genetic interaction between Snf1 and each subunit of the complex, as well as their post-translational modifications as a function of Snf1 activity.

Snf1/AMPK activity is supposed to be responsive to the glucose concentration in the medium. Anyway, some reports indicate that a decrease in the glucose uptake rate, activates Snf1 in glucose repression ^[3]. Because of this, we are now working with strains carrying defective glucose transporters to investigate if the Snf1/AMPK activity could be regulated by the rate of glucose transport rather than by glucose availability.

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