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ABSTRACT BOOK

S01. Intracellular Ca^{2+} dynamics in Vascular Smooth Muscle Cells (VSMCs) isolated from rat pulmonary artery through a non-enzymatic method

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Vascular smooth muscle cells (VSMCs) show a considerable plasticity and can undergo profound and reversible phenotypic changes (from contractile to proliferative) in response to vascular damage or disease, that involves changes in the expression of Ca^{2+} handling proteins, such as SERCA2a[1,2].
The aim of the project was to develop a non-enzymatic method to isolate VSMCs from rat distal pulmonary arteries and characterize their Ca^{2+} dynamics in culture, focusing the attention on SERCA2a function.

The majority of FLUO4-loaded VSMCs showed intracellular Ca^{2+} transients following ATP and high (40 mM) $[K^+]_{out}$ solution superfusion, while only a few cells were responsive to caffeine. SERCA2a and its inhibitor phospholamban (PLN) were expressed. ATP-induced Ca^{2+} transient decay kinetic in Ca^{2+} free extracellular solution was slower under the removal of the contribution of the Na^+/Ca^{2+} exchanger and the PMCA, while it was unaffected by mitochondrial uniporter blockade. Moreover, VSMCs revealed functional microdomains including sarcoplasmic reticulum and mitochondria.
In conclusion, our method permits us to obtain VSMCs expressing the main Ca^{2+} handling proteins in culture, in particular SERCA2 and PLN. The identification of the functional role of SERCA2a in these cells will be crucial to understand its role in preventing phenotypic changes in proliferative vascular pathologies.

Keywords: Smooth muscle cells, SERCA, phospholamban, IP3 receptor, Calcium handling

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S02. Dual role for Dpb4 in DNA double strand break repair: regulation of end resection and DNA damage checkpoint

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The repair of DNA double-strand breaks (DSBs) is essential to ensure genomic stability and to avoid cell death. We show that the highly conserved *Saccharomyces cerevisiae* protein Dpb4 and its interacting complexes ISW2[1] and Polε[2] are involved in the response to DSBs. Dpb4 is known to form histone-like dimers with Dls1 in the ISW2 complex and with Dpb3 in the Polε complex. We found that the deletion of *DPB4* and the *dpb4-A62S* allele identified in our lab cause hypersensitivity to DSB-inducing agents and show negative interactions with the apical checkpoint kinase Tel1. We demonstrate that Dpb4 acts together with Dls1 within the Isw2 ATPase complex to guarantee a histone-free context around the DSB, thus allowing MRX association and the processing of DSB ends. Furthermore, Dpb4 plays an important role in checkpoint activation by interacting with Dpb3 to facilitate the association to DSBs of the checkpoint protein Rad9, which transmits the checkpoint signal to the Rad53 effector kinase. Persistence of both Isw2 and Rad9 at DSBs is enhanced by the A62S mutation located in the Dpb4 histone fold domain that increases Dpb4 association at DSBs. Thus, Dpb4 exerts two distinct functions at DSBs depending on its interactors.

Keywords: DSB, checkpoint, resection, chromatin, *S. cerevisiae*, histones

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S03. Charge patterning is a sequence determinant for protein liquid-liquid phase separation

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Liquid-liquid phase separation (LLPS) is a phase demixing phenomenon in which a supersaturated solution of a macromolecule (protein, nucleic acid) spontaneously generates a two-phase regime in order to minimise the free energy of the system[1]. Phase separating proteins often involve intrinsically disordered regions (IDRs) held as interaction hubs for protein-protein interactions. Among these, electrostatic interactions have been proved to be strongly influenced by both charge density and distribution in protein primary structure[2]. A positive correlation between charge clustering and phase separation propensity has been recently discussed, based on both computational[3] and a few experimental works[4,5].

Herein, a highly-charged IDR from human topoisomerase I has been isolated. Such an IDR includes a 100-residue region (NDR) with regularly alternated cationic and anionic residues and a NLS region holding nuclear localisation signals. Charge permutants of NDR have been designed to enhance charge clusterisation. Synthetic proteins were recombinantly produced and assayed to experimentally prove their disordered nature and their *in vitro* LLPS propensity. Confocal microscope imaging and turbidity assays suggest that charge clustering strongly increases the propensity to phase separate of our model IDR. Overall, data collected so far points to confirm the existence of a correlation between charge patterning and LLPS propensity.

Keywords: phase separation; disordered proteins; charge distribution

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S04. Synthesis of glycan coated nanoparticles for the enhanced active targeting

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Gold nanoparticles are a platform of interest with a broad range of applications, and they are emerging as a powerful tool in nanomedicine[1]. The strong chemical bond among gold and sulfur allows a reliable coating of the nanoparticles' (NP) surface, paving the way to several different bio-applications. In particular, the multivalent presentation of carbohydrates can trigger a cluster effect which can overcome the low affinity of the individual ligands towards their receptors[2]. Moreover, the glycans surface modification can increase the stability of gold NPs in blood, reducing the formation of the so-called "protein-corona"[3] and also preserving their active targeting[4]. In last years, great efforts have been addressed to synthesize and characterize glyco-coated gold NPs, in order to develop reliable and robust nanosystems which can be employed in many fields, from drug delivery to diagnosis[5]. Herein, I undertake two different procedures to obtain a library of ultra-small glyco-gold NPs: 1) A modified Brust-Schiffrin method developed in a bench-top reactor followed by a post-functionalization step to introduce glycans; 2) A new photo-induced one-pot synthesis based on a microfluidic approach: ultra-small gold NPs were synthesized without the addition of template or reducing agents, affording size-controlled functionalized NPs. NPs were fully analyzed by dynamic light scattering and transmission electron microscopy to determine their size and morphology. The interaction between mannose-decorated gold and biological matrices is ongoing and the first results in in healthy mice are very promising.

Keywords: Gold Nanoparticles, Glycans, Nanomedicine

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S05. Phytochemical profile and macrophage immunomodulatory activity of extracts from green and roasted coffee beans

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Interferonopathies are inherited autoinflammatory diseases characterized by a dysregulation of the interferon (IFN) pathway. Type I interferonopathies, such as systemic lupus erythematosus (SLE) and inflammatory myositis, are often resistant to conventional immunosuppressive treatment making it necessary a more effective target therapy.[1] Moreover, type I IFNs have been shown to have negative effects in infections with intracellular bacteria such as *Mycobacterium tuberculosis*.[2] In our study, we characterized the metabolite profile of green (GCE) and medium-roasted (RCE) *Coffea canephora* beans by NMR spectroscopy. Then we investigated the *in vitro* immunomodulatory properties of GCE and RCE in a model of LPS-induced inflammation, using THP-1 and primary $CD14^+$ monocytes-derived human macrophages. Results indicate that GCE and RCE pre-treatment strongly inhibits the release of interferon-β, in a dose-dependent manner, thus suggesting a targeting of the TLR4/TRIF pathway. This effect was corroborated by the inhibition of IRF3 phosphorylation and NF-κB nuclear translocation, observed by immunofluorescence analysis. In addition, we found that RCE is more effective than GCE, suggesting that the roasting process may affect this coffee extract immunomodulatory property. Taken together, our results could open the way to interesting new perspectives to investigate the efficacy of coffee-derived compounds in the treatment of interferonopathies.

Keywords: coffee extracts, macrophages, immunity, inflammation, interferon-beta

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S06. Dissecting the role of the KU complex in the DNA damage response

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DNA double-strand breaks (DSBs) are highly cytotoxic lesions that can form accidentally during DNA replication or upon exposure to genotoxic agents. DSBs must be repaired to avoid loss of genetic information or chromosome rearrangements[1]. Eukaryotic cells can repair DSBs by two main mechanisms: non-homologous end-joining (NHEJ) and homologous recombination (HR). The Ku70-80 heterodimer is rapidly recruited to DNA ends and is involved in these two DSB repair mechanisms[2,3]. To better understand the role of this complex in DSB repair, we searched for *ku70* mutations that suppress the hypersensitivity to DNA damaging agents of cells lacking Sae2, a protein involved in early steps of DSB processing. We identified some *ku70* alleles that restore DNA damage resistance of *sae2Δ* cells. All the mutations are located on an outer face at the N-terminus of Ku70 protein, suggesting that they can alter protein-protein interactions. The characterization of one of these Ku70 mutant variants shows an increase of Ku70 association at DSBs and a suppression of the end-tethering defect of *sae2Δ* cells. The high degree of tethering activity by this *ku70* mutation can explain the suppression of DNA damage sensitivity of *sae2Δ* cells and suggests a role of the Ku complex in maintaining the DSB end closed to each other.

Keywords: Ku complex, double-strand breaks, end-tethering, DNA damage response, *S. cerevisiae.*

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S07. Unraveling metabolic heterogeneity of mammary carcinoma through the development of 2D and 3D culture models

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Breast cancer (BC) is the first cause of cancer-related death in women[1], a highly heterogenic pathology needing a tailored approach to improve treatment responses[2]. Triple-negative BC is the most prone to metastasis and relapses subtype, still lacking a targeted therapy[3]. Metabolic rewiring in cancer is fundamental to maintain the transformed state and survive[4]. Its study represents an opportunity to find new clinical approaches, using 2D and 3D cancer models[5].

We analysed three BC cell lines: SUM159PT, MDAMB231 (both TNBC), and MCF7 (luminal). The measurement of bioenergetic parameters by Seahorse technology consistently predict the response of these cell lines grown in 2D to metabolic perturbations (nutrient deprivation or pharmacological treatments).

The same metabolic treatments were evaluated on spheroids' formation, since 3D cultures better recapitulate the heterogeneity of BC[6]. We observed that glucose metabolism inhibition has the greatest impact on spheroids' formation, especially in the highly glycolytic MDAMB231 cell line.

The results suggest that nutritional and pharmacological perturbations of energetic metabolism have a higher impact on 2D cells proliferation than on 3D cultures formation. In future, these aspects will be evaluated in fully formed spheroids and in heterotypic 3D cultures composed by BC cells and fibroblasts, to better represent the tumor microenvironment.

Keywords: mammary carcinoma, 3D cultures, metabolism, co-cultures, high-content analysis, seahorse technology

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S08. Scalable thermal stimulation as a versatile approach to induce neuronal differentiation

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Heating represents a promising approach to induce cell differentiation or proliferation. In several studies, mild hyperthermia could induce myoblast elongation, muscle atrophy rescue and projection outgrowth [1,2,3]. Moreover, the heat produced by an infrared laser could alter the electrical capacitance of the membrane of mammalian cells [4]. The molecular mechanisms are still unknown, but TRPV channels may be involved in this response.

In this study we used two approaches of thermal stimulation on F-11 cells to verify if heating could modify neuronal behaviour. Preliminary bulk heating experiments were performed to test the best time/temperature combination, the involvement of TRPV channels and the ability of cells to differentiate without differentiating agents in the culture medium. Results showed longer neurites, TRPV1 activity and a significant difference in the electrical parameters recorded by the patch-clamp technique in stimulated cells compared to control. Thus, we investigated the effects of heating at 41,5°C, reached by irradiating Prussian Blue nanoparticles with a near infrared laser. Morphological and functional analysis indicated that stimulated cells were more differentiated compared to the control. These results suggest that a targeted thermal stimulation could induce cell differentiation and support the future application of this method to modify neuronal behaviour *in vivo*.

Keywords: Biophysics, Thermal Stimulation, Electrophysiology, Nanoparticles, Neuronal Differentiation, Neurite Outgrowth

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F01. Development of 3D printed pathophysiological *in vitro* device to study microenvironment effect on glioblastoma progression

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Glioblastoma Multiforme (GBM) is one of the most widespread malignant brain tumor, still representing an important cause of mortality in Europe with a median survival of around 1 year from diagnosis. One of the major actor involved in GBM progression and invasiveness is the differential gradient of hyaluronic acid in the brain microenvironemt at extracellular level. Given the key role of HA in cell mediated ECM remodelling, biomaterials based 3D cell model able to resemble the biochemical and structural properties of GBM are higly desiderable to characterize tumor pathogenesis and combined therapeutic strategies. Here, we report the development of a library based crosslinked hyaluronic acid and collagen or gelatin hybrid hydrogels, employable in the development of GBM 3D tissue cultures obtained by 3D bioprinting. Tunable 3D GBM models with different HA gradient and crosslinking degree have been developed and tested for their biocompatibility with human-glioma U87 cells and then encapsulated in order to obtain an in vitro bioprinted model suitable for high performance predictive screening and studying tumor microenvironment[1, 2, 3].

Keywords: 3D bioprinting, regenerative medicine, hydrogels, tumor

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F02. Overcoming PARP inhibitors drawbacks with a new generation PARP1 selective drug

Phase 1: A novel PARP1 selective inhibitor is effective in preclinical models of ovarian cancer

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PARP – Poly(ADP-ribose)polymerase – inhibitors have demonstrated clinical effectiveness and show greater efficacy in homologous recombination (HR) defective tumours[1]. They inhibit PARP1 and PARP2, as well as other members of the family, potentially responsible for adverse effects, mainly haematological and intestinal[2], which have limited their ability to be combined with chemotherapy.

Here, **PARP1i**, a novel inhibitor selective for PARP1 (meant to optimize the therapeutic windows), was investigated. Patient-derived ovarian cancer xenografts (OC-PDXs) were transplanted subcutis or orthotopically to evaluate the dose response efficacy on tumour growth and dissemination. **PARP1i** was administered orally (0.1-10 mg/Kg) for 8 weeks.

PARP1i did not affect the growth of HOC84 BRCA wild-type tumours. At difference, **PARP1i** stalled the growth of HOC106 BRCA mutated tumours. Most importantly, the anti-tumour efficacy was also evident against two OC-PDXs BRCA mutated yet resistant to Olaparib (one of the PARP inhibitors in clinical use); specifically **PARP1i** inhibited the growth of HOC107 tumours and delayed the dissemination of HOC22, thus significantly prolonging the mice lifespan.

Studies are ongoing i) to investigate **PARP1i** pharmacokinetics and pharmacodynamics and assess the target engagement, in accordance with the drug mechanism of action, and ii) to evaluate **PARP1i** in combination with the standard-of-care treatments for ovarian cancer.

Keywords: PARP inhibitors, Ovarian Cancer Xenografts, Poly (ADP-ribose) polymerase

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F03. Profiling metabolic and signaling phenotype of bladder cancer cell lines and patient biopsies

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Bladder cancer (BC) is one of the most common malignancies worldwide[1]. Most patients are diagnosed with non-muscle invasive BC with frequent recurrences leading to invasive tumors, reducing survival expectations[2,3]. 3D cultures constitute a more clinically relevant model for studying cancer as spheroids recapitulate *in vivo* structure, cell-cell interactions, nutrients and oxygen gradients, absent in monolayers[4]. Metabolic characterization in spheroids could reveal dependence on specific enzymatic activities and nutrients toward novel therapies[5].

This project aims to conduct a comparative analysis of metabolism and cellular features of a BC cell lines panel at different stage/grade, grown as monolayer and spheroids. The obtained 3D models and patient biopsies will be analyzed with raman spectroscopy, for preclinical purposes. Moreover, I will spend six months in Philadelphia, USA, to deepen effects of perturbation of signalling pathways correlated to cancer progression. Samples were analyzed at different timepoints evaluating cellular features by quantitative imaging, bioenergetic parameters by Seahorse technology and validated by WB analysis of key metabolic enzymes. High-grade cells grown in monolayer showed larger use of glycolysis compared to low-grade cells, with no obvious correlation between mitochondrial respiration and grade, or use of respiration and glycolysis during spheroid formation, although this aspect is still under investigation.

Keywords: bladder cancer, spheroids, organoids, metabolism, operetta, raman

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F04. OXPHOS and pharmacological response to PARP inhibition in ovarian cancer

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Ovarian cancer is the most fatal of all female reproductive cancers [1]. The introduction of PARP inhibitors, which exploit homologous recombination (HR) deficiencies to directly target cancer cells, greatly transformed the clinical approach [2]. Recent observations suggested that there is an interplay between HR status and cellular metabolism [3]. In this context, our aim is to investigate the reciprocal relationship between oxidative phosphorylation (OXPHOS) and sensitivity to PARP inhibition. To this end, we evaluated the effect on proliferation and colony formation ability of HR-proficient and deficient ovarian cancer cell lines exploiting the PARP inhibitor Olaparib and an OXPHOS inhibitor.

Consistent with the concept of synthetic lethality, proliferation of HR-deficient cancer cells was strongly impaired by Olaparib. Moreover, the pharmacological inhibition of OXPHOS strongly diminished their proliferation suggesting their reliance on OXPHOS to supply for energy requirements. On the contrary, HR-proficient cancer cells were poorly affected by both inhibitors. Nevertheless, simultaneous OXPHOS and PARP inhibition showed a synergistic effect only towards HR-proficient cancer cells.

These results indicate that OXPHOS perturbation could sensitize HR-proficient cancer cells to PARP inhibition. Molecular studies are ongoing to investigate how the two pathways are interconnected.

Keywords: Oxidative Phosphorylation; PARP Inhibition; Ovarian Cancer

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F05. Platform standardization for the detection of histopathological features in murine models of hepatic and pulmonary disorders

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The clinical progression in both chronic liver and interstitial lung disorders induces parenchymal injury, inflammation and fibrosis. Fibrogenesis is a dynamic process characterized by extracellular matrix production and myofibroblasts activation [1, 2]. Therapeutic approaches still lack because of both several pathological changes and the spread of parenchyma lesions. Mouse models are required to test therapies and a standardized platform to score the degree of fibrosis/inflammation is crucial.
In my first year, I optimized histological procedures to detect inflammation and fibrosis levels from mouse lung and liver sections. To this aim, I combined histopathological methods used in clinic to immunofluorescence assay. I also standardized the acquisition of samples by whole-slide digital image analyses. For fibrosis I used both picrosirius-red and anti-α-SMA immunostaining. For inflammation, I checked the presence of cellular infiltration or microglia/macrophage-specific markers. To assess the integrity of tissues, I counterstained samples with Hematoxylin-Eosin. Since my project is also focused on the correlation between nanocarriers and macrophages, I performed co-localization studies with intranasally injected fluorescent nanoparticles and mouse lung macrophages [3]. This preliminary result will be pivotal for the next steps of my thesis, aimed at evaluating the interaction of nanoparticles with the immune system in liver and lung fibrosis.

Keywords: Histopathology - Nanomedicine - Fibrosis - Inflammation - Nanoparticles

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F06. Is there a biochemical or molecular switch from healthy to symptomatic phenotype in Amyotrophic Lateral Sclerosis?

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Amyotrophic lateral sclerosis is a fatal neurodegenerative disease, characterized by the loss of upper and lower motor neurons at the spinal or bulbar level[1]. MNs loss determines skeletal muscle paralysis and leads to patients death, mostly by respiratory failure[2], and no effective treatment is available. The majority (90-95%) of ALS forms are classified as sporadic (sALS), while about 10% of cases are associated with mutations in specific genes (familial, fALS). Among the numerous defective genes associated with ALS, we focused our attention on two genes that have been identified as definite causes of ALS: SOD1 (Cu/Zn superoxide dismutase-1) and TARDBP (trans active response DNA binding protein), responsible for two distinct familial forms of ALS[3,4]. To further confound the puzzle, independently from the genetic frame, patients display diverse phenotypic expression patterns and may exhibit dramatically different progression rate. For instance, over the past years, we collected fibroblasts from patients with different mutations in SOD1 and TARDBP, but also from healthy donors, belonging to the same family of patients, that have the same mutations of affected individuals but do not manifest any symptoms. This gave us the unprecedent opportunity to investigate the existence of a molecular or biochemical switch from healthy to symptomatic phenotype, thanks to phenotypical, biochemical, and molecular analysis. The relatively poor advancement in understanding ALS pathogenesis reflects the lack of appropriate models. Induced pluripotent stem cells (iPSCs) technology enabled the production of MNs, astrocytes and microglia from ALS patients, allowing both cell- and non-cell-autonomous mechanisms study. So, we are also reprogramming fibroblasts into iPSCs that then will be differentiated to produce MNs, astrocytes and microglia to design *in vitro* co-culture systems with the aim to deepening the understanding of physio-pathological interactions.

Keywords: Amyotrophic Lateral Sclerosis; SOD1, TARDBP; reprogramming; oxidative stress; metabolism.

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F07. Discovery of novel antibiotics from microbes: a project

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Introduction: Even though antibiotic resistance has gained considerable interest over the years, it is not the only way for bacteria to escape antibiotic treatment. Biofilms too are a threat to public health[1], especially since very few antibiofilm drugs are available.[2] Aim: The aim of the project is to discover new antibiofilm molecules, by characterizing over 900 *Actinobacteria* strains belonging to a rare and understudied genus, *Microbispora*, from the Naicons library by paired omics profiling as previously described.[3] Methods: At first, we have to identify and characterize the strains contained in our library belonging to this so-called rare genus by 16S rRNA sequence analysis. We will also determine the culture conditions and extraction methods that allow the highest molecular diversity in strains extracts. Then, a MS fingerprints library will be generated from the extracts analysed by ESI-LC-MS/MS. Using metabolomic and genomic tools to analyze this library, we will identify promising metabolites that will be targeted for purification and structural elucidation. Finally, their antibiofilm activity will be assessed *in vitro*. Material: Out of more than 80,000 strains in our collection, nearly 900 were pre-identified as *Microbispora* on morphological criteria and at least 79 strains were confirmed as *Microbispora* through 16S rRNA analysis.

Keywords: drug discovery, antibiofilm, *Actinomycetes*, paired omics

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S19. Development of a sustainable bioprocess to produce Vitamin B9

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Vitamin B_9, commonly known as folate, is an essential micronutrient that acts as cofactor in one-carbon transfer reactions, therefore involved in many reactions, among which the synthesis of nucleotides and amino acids. Folate deficiency is associated with important illnesses such as anemia and cardiovascular diseases (1). All the vitamin B_9 commercially available is produced by chemical synthesis mainly in the form of folic acid, suboptimal in terms of bioactivity (2). To overcome these current limitations, the main goal of this study is the biotechnological production of bioactive folates using tailored yeast cell factories able to exploit different renewable biomasses as carbon and nitrogen sources. *Saccharomyces cerevisiae* was engineered in the anabolic pathways of the two main building blocks of folate, para-aminobenzoic acid (*p*ABA) and dihydropteridine, to identify the impact on the production of free and poly-glutamate folates. The production of folate from *Scheffersomyces stipitis,* naturally able to grow on different sugars, was tested and compared using synthetic minimal medium, sugar beet pulp (SBP), sugar beet molasses (SBM) and unfermented grape marcs (UGM). In minimal medium the best *S. cerevisiae* strain produced 92,8 ± 5,34 ng/mL of folates, while 214,4 ± 23,39 ng/mL were produced by *wild type S. stipitis*. Moreover, *S. stipitis* was able to produce 101,9 ± 6,62, 189,6 ± 20,9 and 123 ± 8,71 ng/mL, on UGM, SBM and SBP, respectively. The impact of the use of different wild type and engineered strains as well as of different residual biomasses as feedstock will be evaluated in bioreactor fermentations. In the future, techno-economic analysis will be used to evaluate the viability of possible novel value chains, to promote examples of industrial symbiosis.

Keywords: *Saccharomyces cerevisiae*, *Scheffersomyces stipitis*, folate, metabolic engineering, fermentation, residual biomasses, industrial symbiosis

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S10. Convergent technologies in metagenomics: enhancing the available analytical tools

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Metagenomic approaches have changed the way to study biology and biodiversity in several fields. In particular, technology advancement enables us to determine taxa composition and to study complex biodiversity patterns in very different environments. DNA metabarcoding became a standard procedure in recent years, enhancing both fields related to human health[1] and research topics related to the industry, such as food traceability, and ecology, as diet characterization and species associations[2,3]. Considering the huge amount of data produced by researchers and available in repositories, a data mining perspective in managing and exploring DNA metabarcoding data could be useful to collect hidden information and potentially determine undiscovered aspects. In this work, we focused on a data-centered perspective of metabarcoding data, touching three main points that can enhance and ameliorate the use of metagenomics strategies: i) development of new data visualization tools, ii) integration of machine learning and data mining strategies and iii) encouragement of data simulation and data reuse. These three aspects contribute to ameliorate different phases of the metagenomics framework, as the experimental design, the taxonomy assignment, data analysis and data interpretation, with the idea to integrate new methods that can both ameliorate experimental strategies and reveal new aspects related to DNA metabarcoding data.

Keywords: DNA metabarcoding, Bioinformatics, Data visualization, Biodiversity patterns

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S11. A novel combination of synthetic biology approaches towards glucobrassicin production in yeast chassis

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Many microbial pathways are known to lead to the production of industrially relevant molecules. Indeed, microbial cells show several advantages to produce fine and bulk chemicals compared to conventional chemical synthesis, especially for compounds with complex structures. The yeast *Saccharomyces cerevisiae* is a platform of election as a chassis for these purposes[1].

In this scenario, we developed a novel toolkit, which is a combination of synthetic biology approaches. First, we constructed a collection of more than 50 DNA parts, comprising promoters, terminators and sequences coding for enzymes and accessory proteins. These parts were assembled using the Golden Gate Assembly[2], building up a library of expression cassettes with different promoters. These devices can be easily integrated in yeast's genome using the CRISPR-Cas9 system[3]. The system is further improved by promoting the binding of the enzymes to a synthetic protein scaffold, to maintain them in proximity and enhance their efficiency.

To validate the system, we constructed a *S. cerevisiae* strain able to produce Glucobrassicin (GLB), a glucosinolate naturally produced by members of cruciferous vegetables with cancer-preventive properties mainly thanks to its hydrolysis product[4]. We engineered the yeast with genes of GLB pathway from *Arabidopsis thaliana* and screened the best combination obtained by the synthetic biology approach.

Keywords:
Yeast chassis, Pathway engineering, Enzyme clustering, Molecular scaffold

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S12. A multilevel approach to investigate the impact of anthropogenic stressors on pollinator insects across three continents

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Pollinator insects, and so the pollination service, are facing a global decline that highlights the need to better understand how human activities affects pollinators[1] diversity and interactions[1,2]. Here we evaluated how anthropogenic landscape features affect pollinator at multiple levels (species, community, and interactions) in different environmental contexts. To do this, we used field observation, DNA metabarcoding of pollen carried by insects, analysis of pollen deposited on flower's stigmas and GIS land-use characterization. Analysing 264 insects from 26 smallholder farms of Tanzania we found that land-use intensification reduces pollinator richness and increase competition for resource among individuals, while the floral availability manageable by farmers, mitigate this impact. By analysing 332 pollinator insect and 502 stigmas from 11 Maldivian islands we proved that green patches fragmentation, that improve habitat heterogeneity, increase pollinator richness, alters the interaction with plants and thus the pollination efficiency. Finally, we measured wing size and asymmetry of 350 Bumblebee of 2 species, from 37 sites along the urbanization gradient of Milan. Changes in wing size and increased wing asymmetry were observed in urban bumblebees in response to the stressful conditions. These findings increased our knowledge about pollinators, also improving solutions for suitable management and conservation measures worldwide.

Keywords: Bees, Plant-pollinator Interactions, Ecosystem services, Urbanization, Agriculture

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S13. Bowman-Birk protease inhibitor: from phylogenetic diversity to functional efficacy

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Nowadays, natural biodiversity is threatened by many human and environmental factors. In this context, it is fundamental to find promising nature-based matrices that could enhance people's health and enter in the diet of the future. This work is focused on the valorisation of an almost exclusive legume defensive protein, the Bowman-Birk protease inhibitor (BBI). Specifically, its genetic variability and diversity has been investigated in a wide range of *Vigna unguiculata* accessions coming from all over Africa, plus gene sequences submitted to GenBank from *Vigna* and *Phaseolus* genera. The relationships among the found haplotypes were evaluated generating networks and phylogenetic trees, using *Vigna unguiculata* as the core and the other legume species as outgroups. The haplotypes were also compared in terms of protein structure, since different isoforms were found with peculiar amino acid changes. In addition, the isoforms were tested computationally to minimize the interaction energy and find which isoforms are the most promising in terms of binding with their physiological targets, trypsin and chymotrypsin. Since the BBI has been demonstrated to show anti-cancer bioactivity (1,2,3), all these data open the possibility to develop expression systems to produce the purified BBI and so to test it against different cancer cell lines.

Keywords: Bioactive compounds, Bioactivity, Gene evolution, Phylogeny

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S14. Impact of prebiotics and probiotics on gut microbiota and human health

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Nowadays the search for new active molecules leading to beneficial effects for human health is an emerging field of interest. Accordingly, the positive modulation of gut microbiota through the administration of prebiotics, probiotics, or their combination as synbiotics plays a key role. Hence, during the first year of my PhD project, a synbiotic formulation composed of three probiotic strains[1-2-3], and fructooligosaccharides[4] as prebiotics was developed. The resulting modulation of the intestinal microbiota composition and the stimulation of the immune system of healthy elderly subjects[5] were assessed during the second year. The promising results drove the research of the third year to the development of new possible synbiotics through *in vitro* methodologies. The extract from *Grifola frondosa* (Maitake) was identified as a new prebiotic. It is composed principally of ß-glucans, molecules resistant to digestive enzymes, but fermented by the probiotic strains. A probiotic consortium was formulated, and the metabolites produced after Maitake fermentation were collected and analyzed via GC-MSD. Consequently, their effects were tested on healthy and cancerogenic human intestinal cell lines. The results showed a decrease in CACO-2 cell viability, but not in HT-29 and healthy cell line. Furthermore, the metabolites protected the cells from oxidative stress, showing an important viability recovery after the challenge.

Keywords: probiotics, prebiotics, gut microbiota

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S15. Stress-induced ubiquitin-rich aggregates: identification and visualization in the yeast *Saccharomyces cerevisiae*

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By prevailing opinion, protein aggregation represents a dangerous phenomenon, being associated to age-related neurodegenerative disorders, such as Alzheimer's or Parkinson's Diseases[1]. However, this event is not always a synonym of cellular failure or cell death. Indeed, protein aggregation is employed by healthy cells to overcome the presence of large amounts of misfolded polypeptides, generated in response to external stresses, which cannot properly be rescued by the overloaded proteolytic machinery. In this context, Aggresome-Like Induced Structures (ALIS) function as transient deposits for ubiquitinated defective proteins[2].

The yeast *Saccharomyces cerevisiae* is a well-established and powerful model organism to study aging and neurodegenerative disorders, also widely used for the research on cytoplasmic aggregates, including stress granules and processing bodies, as it provided interesting insights on their physiological roles[3]. Therefore, in this study, yeast cells have been engaged to detect the formation of stress-induced ubiquitin-rich structures, identifiable as ALIS-equivalent bodies. To this purpose, cells have been subjected to specific stresses, namely, heat shock temperatures, ethanol administration, nitrogen and glucose starvation. Ubiquitin-rich aggregates were visualized by fluorescence microscopy, observing a higher frequency in cells either exposed to ethanol or under nitrogen starvation. For further investigation, aggregates were isolated under the same experimental conditions and analysed by western blot, revealing a ubiquitin-rich pattern.

Keywords: aggregate, ubiquitin, stress, yeast

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S16. Production and purification of biosurfactants from environmental isolates of fungi and bacteria

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The interest in biosurfactants has been increasing due to many advantages compared with chemical surfactants, including lower toxicity, superior biodegradability, and environmental-friendliness, vast structural diversity and specific activity at extreme conditions (temperature, pH, salinity) and ability to be synthesized from renewable feed-stocks. Amid the COVID-19 crisis, the global market for Microbial Biosurfactants is projected to reach a size of US$18.7 Million by 2027, growing at a CAGR of 3.9% (1).

At BioC-CheM Solutions we have isolated one *Bacillus subtilis* strain able to produce a biosurfactant structurally new but with properties similar to those of the well-known surfactins. Strain improvement followed by fermentation studies allowed the development of a foam-free fermentation process with biosurfactants yields of 10-15 g/L. The purification of the biosurfactant was achieved with a mixed solvent and precipitation extraction. The purified samples are now under study for the identification of the chemical structure and for the assessment of the chemico-physical properties of the new biosurfactant. Fund raising and contacts with companies of the cosmetic sector are ongoing in view of a possible large-scale production and commercialization of the biosurfactant.

Keywords: Biosurfactants, Bacillus, Surfactins, Cosmetics

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S17. Revisiting trehalose production in yeast

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Trehalose, a nonreducing disaccharide, is a valuable commodity with growing interest not only in food and cosmetic industry but also in pharmaceutical applications[1–3]. Currently, the most common production method involves oligosaccharides enzymatic conversion using several enzymes, with a complex purification processes from a mixture of various sugars[4]. The high by-product accumulation becomes the bottleneck of the downstream processes. The global Trehalose market is expected to reach 343.4 million USD by the end of 2026, growing at a CAGR of 5,34% during 2020-2025[5].

With this expectation, an alternative production method using *Saccharomyces cerevisiae* was developed, avoiding the classical starvation and extraction protocol. We started working with a specific yeast strain that is able to redirect fluxes toward trehalose production with a natural secretion ability to external environment. Applying strain improvements and a fed-batch culture strategy a high-purity trehalose can be easily obtained from the fermentation broth. The linearity of secretion during linear feed of nutrient strategy suggests possible extension of production phase as well as further improvements in yield and productivity. In addition, we are working on identifying the correct trehalose transporter that could increase the excreted fraction pushing the process to the limits.

Keywords: *S. cerevisiae*, enhanced metabolism, secondary metabolites, carbohydrates, biosynthetic activities

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F08. Genetic interaction between Mec1 and Mrc1 in response to replication stress

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Replication stress (RS) includes events (i.e. depletion of nucleotides, DNA secondary structures or topological stress) that interfere with replication fork progression and can induce genetic instability, a known hallmark of cancer [1]. Normal cells counteract RS by activating coordinated cellular pathways and the replication checkpoint, which prevents fork breakage, DNA replication completion and mitosis [2]. The replication checkpoint is conserved through the eukaryotes and its key factor is the protein kinase Mec1/ATR. Mec1/ATR recognizes single stranded DNA that accumulates at dysfunctional replication forks and phosphorylates several targets, including the checkpoint mediator Mrc1/Claspin. Mrc1/Claspin in turn triggers the phosphorylation of the effector kinase Rad53 [3]. In addition, both Mrc1/Claspin and Mec1/ATR support unperturbed DNA replication [4]. To better define the crosstalks between Mec1/ATR and Mrc1/Claspin, we explored genetic interactions between different mutant alleles of *MEC1* and *MRC1* genes in *Saccharomyces cerevisiae.* We identified an unexpected positive genetic interaction between Mec1 and Mrc1 in response to depletion of nucleotides, revealing a Mrc1 toxic function in the absence of Mec1. Understanding the molecular mechanism at the base of this interaction could contribute to define the functions of Mec1 and Mrc1 and, since RS is an intrinsic characteristic of cancer cells, could help in the development of therapeutic anticancer treatments.

Keywords: DNA damage, Mec1, Mrc1, replication stress, *Saccharomyces cerevisiae*

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F09. Adverse outcome pathways-oriented toxicology in *in vitro* systems for implementing the safety-by-design of new nanomaterials: preliminary studies on Ag NPs

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Nanomaterials (NMs) are used in a wide variety of commercial products, such as creams, facemasks, protective clothing, and biomedical devices[1]. Silver nanoparticles (Ag NPs) are among the most used metal-based NPs as coating agents due to their effective antimicrobial activity. However, concerns regarding the potential hazard due to the exposure to these NMs pose several open issues about their safe development and use. The aim of this project is to identify the hazard of new Ag-based NMs, designed according to a Safe-by-Design (SbD) approach, toward human health during their production and use. Furthermore, an adverse outcome pathways (AOPs)-oriented testing strategy is being developed, in order to connect NPs physico-chemical (p-chem) properties and the mechanistic aspects of their biological reactivity to the potential health effects in humans and other organisms[2]. In this initial study, after developing a harmonized protocol for the preparation and characterization of the NP suspensions, their p-chem properties were evaluated by TEM, DLS and UV-vis. The cytotoxic effects on the human lung cell line, A549, of different Ag NPs – naked (Ag-NKD) and coated with polyvinylpyrrolidone (Ag-PVP) or hydroxyethyl cellulose (Ag-HEC) – were evaluated. The results on viability, cell death mechanisms, inflammatory response, oxidative stress, and NPs uptake demonstrate that the cellular responses to the AgNPs strictly depend on their p-chem properties, and in particular on the properties of the coating polymer. These results are useful for implementing the safety-by-design strategy in the framework of the ASINA project.

Keywords: silver nanoparticles, safe by design, adverse outcome pathways, in vitro toxicity, inhalation toxicity

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F10. Development of innovative green procedure for the recovery of active compounds from food and agricultural by-products

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The work is part of the CHRONOS project that studies the mechanisms behind chronic non-communicable diseases (NCDs). NCDs depend on genetic, psychological and environmental factors able to reduce or increase the risk of these diseases. Among these environmental factors, diet also plays a significant role. Food is indeed a source of nutrients, but also of many non-nutritive molecules that could have effects on human health[1].

In this framework, starting from agricultural food products and by-products, we have developed different procedure to extract, characterize and evaluate the bioactive molecules. In the first part of project, we have focused our attention on four by-products, artichoke leaves, cocoa shells, spent seeds of *Camelina sativa* and by-product of orange fruit. After the extraction, analyses by UPLC-DAD-ESI-QTOF-MS showed the presence of several metabolites belong to different classes as: polyphenols, methylxanthine and glucosinolates.

In the future, it is planned to optimize the extraction process by means of green procedures such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) to improve the extraction yield reducing time and solvent consuming[2]. Additionally, we would like to investigate the presence of potential contaminants or xenobiotics such as mycotoxins[3], pyrrolizidine alkaloids that could play harmful effects to human health.

Keywords: mass spectrometry, polyphenols, *Theobroma cacao*, *Cynara cardunculus*, pressurised liquid extraction, supercritical fluid extract

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F11. Unraveling the impact of urbanization on pollinator insects and their influence on fruit chemical features

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This research project aims at establishing a comprehensive knowledge of the impact of anthropogenic pressures on the provision of pollination ecosystem service, mediated by insects, through a multidisciplinary approach. These investigations were triggered by a previous study showing how urbanized environments could drive a shrinkage of body size and an increase in wing asymmetry in two species of bumblebees (*Bombus* spp.), suggesting a putative impairment of their pollination efficiency [1]. To better elucidate the root causes of these phenomena we focused on investigating the chemical features of floral resources by sampling pollen and nectar from five and three plant species respectively in 16 sites along an urbanization gradient in the metropolitan area of Milan. The aim was to analyze the protein/lipid ratio, as well as the occurrence of secondary compounds known for their biological activities on insects through UPLC/MS^n approach. Furthermore, the role of pollination in determining nutraceutical properties of agronomic products will be evaluated by inducing different pollination treatments on 120 plants of *Fragraria vesca* L. The outcomes of this multi-disciplinary approach will shed new light on the interplay between the environment and human health, paving the way for efficient policy actions to achieving the *one health* goals.

Keywords: Pollination ecology, Phytochemistry, Landscape anthropization, Bumblebee, Geometric morphometric

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F12. Role of the DNA damage response in R-Loops recognition and signalling

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DNA transcription and replication are essential physiological processes that can turn into a threat for genome integrity when they compete for the same DNA substrate. During transcription, the nascent RNA binds the template DNA strand, leading to the formation of an RNA-DNA hybrid structure and generating a single-stranded DNA, forming a so-called R-loop. R-Loops plays several physiological roles[1], but their deregulation is clearly representing a source of DNA damage and genome instability[2]. Then, a better understanding of how R-Loops generate DNA damage and activate DNA damage response (DDR) is needed. Recently, the highly conserved MRN/MRX complex was shown to suppress R-loop formation genome-wide both in yeast and mammals. However, the role of its partner ATM/Tel1 kinase is still unclear[3]. We observed that, in yeast, mutant cells accumulating R-loops are more sensitive to DNA damaging drugs and replication inhibitors when Tel1 is absent. Interestingly, by measuring RNA-DNA hybrid levels through DNA-RNA Immunoprecipitation (DRIP)[4], we found out that the lack of Tel1 does not cause an increase in RNA-DNA hybrids, even in R-Loop accumulating cells. In addition, by exploiting specific MRX mutants that impair MRX-Tel1 interaction, we showed that the MRX complex is involved in the role of Tel1 at R-Loops. In this complicated scenario, it will be important to understand whether Tel1 is required to limit R-loop-derived damage/genome instability and the molecular mechanisms behind this role.

Keywords: DNA damage, R-Loops, RNA-DNA Hybrids, *Saccharomyces cerevisiae*, ATM

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F13. New perspectives for textile waste: recycling material by mechanical/chemical pre-treatment and enzymatic hydrolysis processes

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The fashion sector usually relies on a linear business model from the economic point of view. Large amounts of non-renewable sources are exploited to produce clothes: because of a short turnover, they quickly become textile waste. Today, less than 15% of clothes are collected for recycling and less than 1% of the fibres is recycled into new ones[1].

The most common way of recycling textile is the mechanical one. Textile wastes are mechanically destroyed and then spun. However, this process can be easily applied to natural fibers but not to mixed ones. Furthermore, the quality is reduced, while costs increase simultaneously. Therefore, this research project aims to investigate a biotechnological valorisation of textile wastes.

The European Community strategic objectives are focused on transforming the production system, albeit this implies important scientific and technological challenges. The project aims at the treatment of industrial textile wastes by enzymatic hydrolysis in association with mechanical/ chemical pre-treatments to reduce the fibers to the constituent units. Then, glucose obtained from cotton can be reused in industrial sectors like the textile itself, supporting industrial synergy with a circularity principle[2].

In addition to the specific results, the return of the project is the establishment of a circular bioeconomy model, based on sustainable development and the creation of a biobased supply chain.

Keywords: Microbial biotransformation, Circular economy, Bioeconomy, Sustainability, Biobased Supply Chain

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F14. Development and characterization of vault-based nanocarriers in *Pichia pastoris*

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Vault is the largest known ribonucleoprotein particle, naturally occurring in higher eukaryotic cells[1,2]. Vault is involved in several cellular functions. Many of its features, such as its non-immunogenic structure and its huge internal cavity, make it a suitable nanovector for drug delivery[3]. Recombinant vaults are produced by expression of the major vault protein, occurring in 78 copies that assemble into a barrel-like "nanocapsule".

Baculovirus-insect expression is the most used system for recombinant vault synthesis. However, it suffers from low scalability and slow production rates. Thus, the yeast *Pichia pastoris* (reported to enable vault expression at lower costs and in higher yields[4]) has been chosen to constitutively express human recombinant vaults. Recombinant vaults from *Pichia,* purified by size exclusion chromatography followed by ultracentrifugation, display same morphology and size of authentic vault as shown by transmission electron microscopy, dynamic light scattering and NTA analysis.

Vault engineered variants containing a Cys-rich stabilizing domain or carrying a membrane-lytic peptide enhancing endosomal escape have been constructed. Their characterization and the construction of a variant allowing antibody-direct conjugation is in progress. Future steps, involving vault-mediated targeting of active molecules to specific cancer cell lines, will be conducted at the *Université de Paris* according to *cotutelle* agreement.

Keywords: vault protein, nanocarrier*, Pichia pastoris,* protein engineering, recombinant expression

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S18. Cross-linking effects dictate the preference of galectins to bind LacNAc-derived HPMA copolymers

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HPMA copolymers loaded with carbohydrate ligands have been recently proposed as efficient inhibitors for lectins[1], showing interesting possibilities as drug-free nanomedicine devices for cancer therapy. Herein, the interaction of combined poly-LacNAc (Galβ1-4GlcNAc)-containing
N-(2-hydroxypropyl) methyl acrylamide (HPMA) copolymers (and the respective building blocks) with human galectin-1 and the carbohydrate recognition domain (CRD) of human galectin-3 has been analyzed by NMR methods, assisted by electron-microscopy and DLS experiments. For galectin-3 CRD no evidence for forming a large supra-molecule and no preferences for the different multivalent presentations of the glycopolymers have been detected. In contrast, for galectin-1, the results indicate the formation of large cross-linked supramolecules in the presence of multivalent LacNAc entities, for both the individual building blocks and the polymers. In the case of the polymeric entities, large supra-structures were generated in the presence of sub-stoichiometric amounts of LacNAc epitopes. Interestingly, the bivalent and trivalent presentation of LacNAc in the polymer did not produce such increase, indicating that the multivalency provided by the polymer is sufficient for triggering an efficient binding between the glycoconjugate and galectin-1. This hypothesis was further demonstrated by electron microscopy and dynamic light scattering methods[2].

Keywords: galectin, multivalency, glycomimetics, molecular recognition, HPMA copolymer, inhibition, glycopolymer

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S09. Osteoarthritis theranostics: characterization of synovial fluid extracellular vesicles and an osteoarthritic joint-on-chip model

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The extracellular vesicles (EVs) emerged as innovative potential biomarkers as well as promising therapeutic agents for osteoarthritis (OA) [1], a pathology characterized by the lack of early markers and effective therapeutic approaches [2,3].
Aiming to develop an OA joint-on-chip model as screening platform for innovative biological treatments, I optimized the culture conditions within the microfluidic model developed in the past years. I produced and compared different hydrogels to culture the chondrocytes in an environment better resembling the in vivo one. In the future, this model could be used to compare the effectiveness of innovative biological approaches, such as EVs, in counteracting the OA.
Parallelly, I developed a separation method to isolate EV subpopulations with different size from the synovial fluid. Based on the asymmetrical flow-field-flow fractionation, it allowed the separation of different-sized EV subpopulations within a wide size range (40-1400 nm in diameter). Once isolated, I characterized and compared the EV subpopulations from arthritic and non-arthritic synovial fluid by assessing the size, the total protein and nucleic acid content, the Z potential, the surface markers and by performing TEM imaging and lipidomics analysis.
During the last part of the project, I will perform proteomic analysis of the cargo of EV subpopulations.

Keywords: Osteoarthritis, Extracellular vesicles, EVs, EV subpopulations, joint-on-chip, microfluidics

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S20. Synthesis and characterization of cell-derived biomimetic nanoparticles and their potential for the investigation of Cancer Associated Fibroblasts (CAFs) function

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The increased awareness of stromal contributions to cancer progression has led to a new cancer treatment paradigm: targeting the tumor stroma acquire primary role in the fight against cancer[1,2]. Tumor microenvironment (TME) plays a key role in assisting tumor progression and resistance[3,4]. Among TME components, cancer-associated fibroblasts (CAFs) are the most abundant population and represent a pivotal player in TME modulation[5,6].Taking advantage of this, together with CAFs perivascular localization, we propose an innovative therapeutic strategy consisting in reprogramming CAFs by means of gene therapy to kill cancer cells. Our approach relies on the delivery of TNF-related apoptosis-inducing ligand (TRAIL) gene in CAFs using cell membrane-derived biomimetic nanoparticles (CMNPs). These are obtained by decomposition of the target tumor cell membranes, exploited to promote the interaction with CAFs. After cell membranes isolation, CMNPs are obtained through extrusion and characterized in morphology (size and surface charge) as well as in their protein content to assess the retention of membrane proteins responsible for TME tropism. Next, plasmid encoding the tumor-specific apoptotic cytokine TRAIL will be loaded onto CMNPs which will serve as CAFs-targeted vectors. Simultaneously, preliminary transfection studies have been conducted using TGF-beta activated NIH3T3 fibroblasts to resemble CAF population.

Keywords: biomimetic nanoparticles; cell membranes; tumor microenvironment; cancer associated fibroblasts; fibroblast-CAF transition

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S21. Multimodal nanoparticles for *in vivo* imaging of pancreatic islets

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In vivo imaging techniques allow us to investigate within organisms targeting specific biological markers. This technology could find crucial applications for some pathological conditions. In particular, the targeting of pancreatic β-cells is acquiring tremendous interest, since it reveals precious information regarding the cell viability, and developing an efficient imaging approach can find applications in regenerative therapies for diabetes and early pancreatic cancer diagnosis.
Nanoparticles are a promising candidate as versatile probes, since their surface is ideal for labelling both targeting and contrast agents. In this work, nanoparticles for multimodal imaging have been designed based on the combination of two polymeric components, chitosan and γ-PGA, and formulated as self-assembled polyelectrolyte complexes. The polymeric components have been functionalized for subsequent chemoselective decoration with a ligand for specific targeting of β-cells and different detecting agents, exploiting multiple imaging techniques (PET, SPECT, MRI, MSOT).[1] The composition and the purity of polymers have been verified with different analytical methods. The properties of the nanoparticles have been characterized, and the biocompatibility of both polymers and nanoparticles was examined in vitro and in vivo. Biodistribution has been tested by PET in mice with Ga-68 labelled nanoparticles.

Keywords: GLP-1R targeting, beta-cells targeting, Exendin-4, multimodal imaging, diagnostic nanoparticles, Pancreatic Islets

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S22. Convergent technologies on eukaryotic and prokaryotic central carbon metabolism

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Biological systems robustly evolved to adapt to internal and external perturbations. This is achieved through a thick network of interactions between metabolism and signal transduction pathways, which result in the fine-tuned coordination of between cell metabolism, cycle and growth. This concept, highlighted by the last decade progresses in system biology, is nowadays central in the study of mammalian and microbial metabolism for fundamental and applied purposes. The molecular interaction between metabolites and regulatory proteins (PMIs) is a widely conserved mechanism by which such a coordination is achieved[2]. As example, glucose inactivation of the Snf1/AMPK kinase in the yeast *Saccharomyces cerevisiae* has been proved to be independent from Ras/PKA pathway opening to the hypothesis of a direct connection with glucose metabolism. The complexity described above is also at the base of the unexpected influence of methionine metabolism on the activity of Snf1/AMPK in both yeast and mammalian cells [3,4], whose comprehension opened to a newly treatment of liver cancer combining methionine restriction and AMPK inhibition.
Furthermore, metabolic robustness has been proved to be an extraordinary resource for synthetic biology purposes, even if a comprehensive knowledge of metabolic network of the host organism is essential for a rational engineering.

Keywords: Glycolysis, TCA, Snf1/AMPK, PKA, S-Adenosylmethionine, *Saccharomyces cerevisiae*, liver cancer, *Pseudomonas putida*

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S23. The advantages of microfluidics technology for nanoparticles continuous manufacturing designed for oral delivery systems

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Oral nanoparticles (NPs) have been selected as an alternative approach to improve the solubility and the stability of active ingredients in the gastrointestinal tract. The nanocarriers could reach both local and systemic drug targeting; allow a control release of encapsulated drugs, reducing the frequency of administration; and ameliorate patient's compliance [1]. In order to allow the nano inclusion in oral dosage forms and promote the clinical translation [2], it is necessary to develop a scale up protocol from single synthesis of NPs to continuous manufacturing production. One of the technologies successful for this purpose is the microfluidics system that guarantees a higher yield of process, provides a better reproducibility, and ensures an increase of drug encapsulation efficiency[3].
In this scenario, this project started with the setting up of some protocols using microfluidic systems, both for polymeric and lipidic nanoparticles, which will be included in different oral delivery systems used on market (tablets, pellets, "printlets"). In particular, polymeric NPs loaded with Indomethacin, a low soluble drug used as tracer, have been produced and its stability and the release of loaded drug in biological fluids will be studied.

Keywords: oral delivery, microfluidics, nanoparticles, liposomes

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S24. Biopolymer-based hydrogel as multifunctional 3D printable bio-ink for tissue engineering applications

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Tissue engineering evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues.[1] The glycosignature of ECM is particularly important to control cell fate, regulation and tissue physiology.[2] Glycans are expressed in the ECM as polysaccharides in glycosaminoglycan (GAGs) and proteoglycans (PGs) and as oligosaccharides in glycoproteins. To closely mimic the ECM in 3D structures, several polysaccharides or oligosaccharides have been employed and combined with proteins to obtain hybrid scaffolds. An example of polysaccharide with high biomedical potential is chitosan, a linear polysaccharide formed by β-1,4-linked d-glucosamine (GlcN) and N-acetyl-d-glucosamine (GlcNAc) epitopes. Chitosan is FDA approved and has outstanding properties such as excellent biodegradability, biocompatibility and antimicrobial activity.[3] In this work, elastin, gelatin and chitosan, as proteinaceous and polysaccharidic natural polymers, have been functionalized with methylfuran groups to allow crosslinking with 4-arm-PEG-maleimide, in order to obtain stable and tailorable matrices for the validation of 3D bioprinted models. The mild reaction conditions of the Diels Alder crosslinking allowed the U87MG glioblastoma cell encapsulation in the 3D-bioprinting process, without affecting cell viability.

Keywords: Bioprinting, Tumour model, Hybrid Hydrogel, Diels-Alder reaction.

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ABSTRACT BOOK
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Prof. Paolo Soda graduated with honours in Biomedical Engineering at Università Campus Bio-Medico (UCBM), Rome, in 2004 and received a Ph.D Biomedical Engineering (Computer Science area) in 2008 from the same University. Currently he is Associate Professor of Computer Science and Computer Engineering at UCBM, and he is co-responsible of the Collaborative Laboratory of Precision Medicine & BioData Analytics between UCBM and Centro Diagnostico Italiano, Milan.
His research interests include artificial intelligence, pattern recognition, machine learning, big data analytics, and data mining applied to data, signal, 2D and 3D image and video processing and analysis. Practical applications of the research activities have impacted on the biomedical applications, with reference to computer-aided diagnosis and decision support systems. He has been co-PI of several national and international projects. His research activity is certified by >110 scientific publications, >1400 overall citations, h-index 20 and an i10-index 41. He is co-author of four conference awarded papers (IEEE LSC 2018, IEEE BIBM 2018, IEEE ICCI*CC 2019, IEEE CBMS 2021). He led the team winning the competition "All against COVID-19: Screening X-ray Images for COVID-19 Infection", IEEE, 2021. He is a member of the IEEE, CVPL and SIBIM, and he is chairing the IEEE Technical Committee on Computational Life Sciences.

T01. Artificial Intelligence and Machine Learning: an introduction

Artificial Intelligence (AI) is the science and engineering of making computers behave in ways that, until recently, we thought required human intelligence [1]. In other words, it is the ability of computer systems to perform tasks normally requiring human intelligence, such as visual recognition, speech, translation, being a branch of computer science that learns and solves problems. Machine Learning (ML) is a subset of AI that uses data to learn and to automatically solve predictive tasks. These solvers are trained models of data that learn based on the information provided to them. This information is derived from probability theory and linear algebra. Within this umbrella, Deep Learning is a subset of ML which relies on multilayered neural networks to solve these tasks. In this talk we will review these main concepts, understanding the three types of AI, the different types of learning even though the discussion of some toy examples. An overview of the learning issues will also be presented.

References

1. Andrew Moore, Former-Dean of the School of Computer Science at Carnegie Mellon University.

T01-second part.
Bio-image informatics: empowering humans' skills by machine learning

High throughput medical images are now used in different areas of medicine to detect disease, to plan treatments, to follow up the patient, etc. The quantitative nature of the images allows us to go beyond visual interpretation by computing, analysing and selecting advanced quantitative imaging features. This in turns has led to bioimage informatics, that has been permitted to empower humans' skills by developing machine learning-based methods and tools able to support the decision process. Indeed, it is now possible to extract and mine quantitative information from the data at different scales. In this lecture we will overview this exciting research topic, introducing some applications developed in our laboratory that help understanding the potential of AI in this field.

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Dr. Eduard Porta-Pardo obtained his degree in Biotechnology from Universitat Autonoma de Barcelona in 2008 and his PhD in Biomedicine from Universitat de Barcelona in 2013. Between 2013 and 2017 he was a postdoctoral associated at SBP Medical Discovery Institute (La Jolla, USA) where he worked on how to use of protein structure data to identify cancer driver genes and drug biomarkers.

Between 2016 and 2018 he co-led two analysis working groups from The Cancer Genome Atlas PanCanAtlas project, on cancer driver genes and oncogenic mechanisms, and contributed to a third on the immune landscape of cancer. Between 2018 and 2019 he was a La Caixa Junior Leader Fellow at the Barcelona Supercomputing Center (BSC). There he worked on how germline variants shape the immune response against tumors and on the use of polygenic scores to predict cancer risk.

Since 2020 he leads the Cancer Immunogenomics laboratory at the Josep Carreras Leukemia Research Institute. The group uses computational approaches to understand how genetic variants, both germline and somatic, interact with the tumor microenvironment, particularly with immune cells.

T02. Personalized cancer risk prediction with polygenic risk scores: clinical and biological implications

Over the last 15 years we have identified hundreds of inherited variants that increase the risk of developing cancer. Polygenic risk scores (PRS) summarize the genetic risk of each individual by accounting for the unique combination of risk alleles in their genome. In the case of cancer, one of the potential uses of PRS in the clinic is the identification of individuals that have higher or lower than average genetic propensity to different types of cancer.

So far, most studies of PRS in cancer have focused on their predictive value in the overall population. However, cancer is a very heterogeneous disease, and little is known about how PRS correlate with clinical variables: from before the tumor actually develops, to the somatic properties of the primary tumor. Understanding the relationship between PRS and these variables is fundamental to predict the potential consequences of the widespread use of PRS in the general population.

Here we used data from two large cohorts, the UK BioBank (UKBB) and The Cancer Genome Atlas (TCGA) to study the correlation between PRS and all these variables. We found that the prevalence of many types of cancer for people at the top 5% of the genetic risk strongly depends on other known risk factors. We also show how PRS for some cancers correlate with molecular subtypes or cancer driver events. Our results highlight important questions that could improve the predictive power of PRS and that need to be answered before their widespread clinical implementation.

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Giulia de Martini is Head of Research at TheFabLab, a digital manufacturing laboratory based in Milan, where she fosters innovation and studies its impact on society. Graduated in Physics and Biostatistics with a master degree in Science Communication, she spreads awareness about the technological and human competences involved in the Fourth industrial revolution, focusing on the connections between different fields of knowledge.

After a decade of experience in the world of clinical research, she founded SPARK Art & Science, to study relationships between technology, science, art and society.

In her former position at the National Museum of Science and Technology in Milan she performed her research in the area of Visitors Studies and Evaluation.

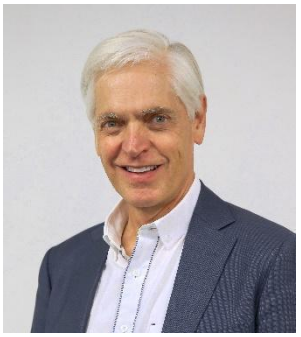
In 2020 she co-edited the volume “Art in Science Museum - Toward a Post-Disciplinary Approach” published by Routledge, London.

T03.HUMANS DISEGN TECHNOLOGIES and TECHNOLOGIES DESIGN HUMANS

Technologies play a great role in human's history, from the governance of fire and the first flint instruments to Social Network and Artificial Intelligence, the evolution of our species has always been characterized by our relationship with technology. Actually, it is not just a relationship it is rather a symbiosis. Our species is the result of the co-evolution of biology and technology.

Unfortunately, we think devices, instruments and machines are just like technical matter instead driver of our humanity…In the XXI century, it's important to realize that scientists and technicians designing and producing technologies have a big responsibility for our future.

Isak S. Pretorius



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Isak (Sakkie) Pretorius is Deputy Vice Chancellor Research at Macquarie University in Sydney, Australia. He has a background in wine biotechnology and synthetic yeast genomics. Sakkie began his career in South Africa. At Stellenbosch University, he became the founding Director of South Africa's Institute for Wine Biotechnology. In the US and Europe, he conducted research at the Albert Einstein College of Medicine in New York, the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, and the Katholieke Universiteit Leuven in Belgium. In 2003, Sakkie relocated to Adelaide to take up the role of Managing Director of the Australian Wine Research Institute. In 2013, he took up his current role as Deputy Vice Chancellor Research at Macquarie University where he also initiated a research program in Synthetic Biology and founded the Australian Genome Foundry. He is the driving force behind a university-wide BioInnovation Strategy and leads the Australian team of the International Synthetic Yeast Genome project.

T04. The convergence of synthetic biology and artificial intelligence

Synthetic Biology has revolutionised research in life sciences - initially as a game-changer in molecular biology, unfolding a wide set of highly precise tools from routine laboratory tasks (e.g., single gene synthesis or editing) to modifying complex metabolic circuits. By combining DNA reading (sequencing), writing (synthesis) and editing technologies with the integrative functionalities of information technologies, artificial intelligence, machine learning and biodigital engineering, it seems reasonable to envisage a future scenario where the practices of synthetic biology are being integrated into multiscale designs enabling two-way communication across organic and inorganic information substrates in biological, digital and cyber-physical system integrations. Novel applications of bio-informational engineering will arise in environmental monitoring, precision agriculture, precision medicine and next-generation biomanufacturing. Potential developments include sentinel plants for environmental monitoring and autonomous bioreactors that respond to biosensor signalling. As bio-informational understanding progresses, both natural and engineered biological systems will need to be reimagined as cyber-physical architectures. It is expected that multiple length scale taxonomy will assist in rationalizing and enabling this transformative development in engineering biology. These platform capabilities will not just enable new approaches to old and intractable challenges, they stand to transform the ways in which research is conducted and societies operate.

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I graduated in Economics at Bocconi University (Milan), and Political Sciences at Milan State University. I taught Communication Sociology at Milan State University for many years. While working there, I also headed the Communication Research Department of CRA, the Nielsen ad hoc research institute in Italy.

In 1995, I felt I wanted to be independent and left the academy. I founded Alphabet, a boutique Milan-based research agency which specializes in market research and communication analysis.

Now I am a sociologist, semiotician, brand and storytelling expert. In my market research activity I have worked for the most important local or multinational companies doing business in Italy. I also conducted extensive research in the US, several European countries and Asia (Japan, China, Thailand).

A few years ago I started to teach again: I am part of the faculty at the Master in Marketing Utilities and Storytelling Techniques, Pavia University.

In the last decade I wrote several books with some colleagues or by myself. Those that I wrote independently are titled: “Viral Stories” (Lupetti 2012), “Web Storytelling” (FrancoAngeli 2018), “Storytelling and Artificial Intelligence” (FrancoAngeli 2019). All deal with communication subjects in the age of the web and new technologies.

T05.About *Storytelling* and *Artificial Intelligence*

It all started when I met this sentence by Elie Wiesel: *“God created man because He loves stories”.*

What this phrase implies is that stories are intrinsically associated with the human condition and are, perhaps, the best humanity can offer. But I began to think… does this concept still hold at the time of AI? That was when I decided to write my book *Storytelling and Artificial Intelligence*.

Storytelling has always been the exclusive domain of man. No other living creature has ever demonstrated this particular inclination, linked to human capacities such as symbolic elaboration, language, creativity.

From now on, however, storytelling may no longer be a purely human endeavour. The current transition is leading artificial systems to discover the secrets of narratives in a number of crucial areas of contemporary communication: cinema, journalism, marketing, advertising, politics.

A whole new era is thus opening up. Clearly, it is mainly determined by the impressive development of artificial intelligence in a multitude of fields. But the fact that AI will soon be able to catch up with us in terms of storytelling - and making sense of the world - means that this world might never be the same again.

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Professor of General and Social Pedagogy, President of the Italian Universities' Network for Lifelong Learning and joint convenor of ESREA's Life History and Biography Network, her main research interests are in adult education and learning, with a special interest for transformative theories. She developed a systemic compositional approach to adult education research and intervention, mixing participatory and arts-based methods with (auto)biographical writing, embodied experience and critical reflexivity. Her book *Transforming Perspectives in Lifelong Learning and Adult Education: A dialogue,* written with Linden West received the 2019 Cyril O. Houle Award from the American Association for Adult and Continuing Education.

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Researcher in General and Social Pedagogy, his research interests are connected to lifelong learning and mainly focused on transitions in learning and professional careers. He explores these themes through auto/biographical methods and from systemic and constructionist perspectives. He is joint convenor of two ESREA (European Society for Research on the Education of Adults) research networks: "Access, Learning Careers and Identities Network" and "Working Life and Learning Network". He is also joint convenor of the network "Research in Higher Education" of EERA (European Educational Research Association).

T10. Developing a professional identity along the PhD training: issues, struggles, possibilities

The “doctoral journey” (Taylor et al. 2011) is a complex and challenging experience that builds many kinds of knowledge, attitudes, skills, and values. The whole person is involved, and the meaning and outcomes of this life-changing experience go far beyond the mere acquisition of expertise and research skills – such as learning about specific fields, research methodologies, knowledge of specific objects and questions, and becoming socialized to a specific scientific community. Struggles of identity are quite common in the PhD path: who am I? Do I really want to be a researcher? And why? Is there a place for me in the scientific community? In the academy? Or outside? Educational research has shown the importance of developing a narrative about one’s own identity as an early career researcher, knowing all the contradictions and ambiguities of the context. In fact, professionalization of research, increasing competition, and constant evaluation bring struggles and frustration that deserve self-reflexivity in order to develop better knowledge of one’s own potential, values, and capabilities.

The workshop will propose self-reflexive exercises, using personal narratives to trigger a conversation about emerging issues, struggles and possibilities for the future. The facilitators will share their research and experiences in PhD training, to sustain a collective and dialogic experience of critical learning.

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