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S01.Remote thermal stimulation: a scalable method to induce cell differentiation

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Heating represents a promising approach to induce neurite outgrowth and neuronal function recovery. In previous studies, protocols with different temperatures and durations[1,2] induced differentiation of different cell types like cultured mammalian cells, neurons, stem cells and cancer cells. This effect has been attributed to changes in cell membrane capacitance and in ion channel properties[3], but the underlying mechanism remains so far unknown. By inducing a scalable thermal stimulation we investigated the eventual modifications on the behaviour of F-11 cells, a model of dorsal root ganglion neurons[4]. Morphological and electrophysiological analysis were performed to characterize neuronal differentiation. Culture medium temperature was increased to 41.5°C for 30 minutes by irradiating a disk of Prussian Blue nanoparticles, placed on the outer floor of the petri dish, with near infrared laser. Cells at 37°C were used as a control. Neurite elongation in irradiated cells was significantly increased compared to control cells and significant differences were also observed in resting membrane potential, action potential firing frequency and spontaneous activity recorded by the Patch-Clamp technique. These results suggest that a targeted thermal stimulation could be an encouraging approach to induce cell differentiation and support its future application as a strategy to modify neuronal behaviour in vivo.

Keywords: Dorsal Root Ganglion neurons, Patch-Clamp, Electrophysiology, Bulk Heating, Neuronal Differentiation, Neurite Outgrowth

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S02. African leafy vegetables as sustainable source of bioactive phytochemicals and micronutrients.

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Leaves of African vegetables belonging to the *Vigna*, *Lablab*, *Corchorus*, *Amaranth*, *Cleome* and *Solanum* genera are known to be very rich in secondary compounds and micronutrients and have been recommended for alleviating food and nutrition insecurity in sub-Saharan Africa[1,2]. In this work we evaluated if the phenolic composition of *Corchorus olitorius* leaves is influenced by different agronomic practices such as minimum tillage and water scarcity and if the prevention ability against colorectal cancer (by using Caco-2 cells model) is elicited by certain growth conditions. We found comparable levels of antioxidants in leaves grown under different agronomic treatments and a tendency to show higher colorectal cancer prevention abilities from plants grown under no tillage management. The mechanism through which *C. olitorius* leaves act against the cancer cell line deals with an increase of the total ROS amount and with an inhibition of the glutathione-independent antioxidant enzymes. At the same time, the amount of different vitamin B9-derivatives was assessed by HPLC-DAD. Results showed that *Cleome gynandra, Lablab purpureus, Vigna unguiculata* and *Corcorhus olitorius* were richer in folates (6.5-33 mg per 100 g fresh leaves) than other common cash crops such as beetroot or common spinach.

Keywords: Leafy crops, jute mallow, African indigenous vegetables, phytochemicals, micronutrients, bioactivity

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S03. A novel in-vitro human model to study and treat aging-induced cardiac dysfunction

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Aging of the heart involves adverse remodelling in cardiomyocytes (CMs) which results in heart failure with incidence that increases with age[1]. Interestingly, till now we lacked a human model of cardiac aging.

This study exploits CMs from human induced pluripotent stem cells (hiPSCs) to reproduce and characterize mechanisms involved in aging and test cardioprotective therapies, like through cardiac progenitor cell (CPC)-derived exosomes (EXO)[2]. Human CPCs were reprogrammed into hiPSCs and subsequently differentiated in hiPSC-CMs. A senescence-like phenotype (SL-CMs) was induced by short exposure (3 hours) to doxorubicin (DOX) at sub-lethal concentration (0.2 μM). 24h following DOX treatment, SL-CMs were exposed to EXO (~$2 \cdot 10^3$ particles/cell) collected from culture media of CPCs by ultracentrifugation.

DOX treatment induced senescence, as confirmed by activation of p21 and increased SA-β-gal positivity compared to CTR. SL-CMs showed impaired calcium-handling and prolonged QTc vs CTR due to reduction of I_{Kr} current. Biochemical analysis revealed presence of oxidative stress and a depolarized mitochondrial membrane potential which resulted in decreased ATP production by mitochondria. These effects were mitigated by exposure to EXO.

Overall, SL-CMs recapitulate the phenotype of aged CMs in terms of senescence markers and electrical and metabolic properties. Additionally, exposure to CPC-derived EXO limited age-related cardiac anomalies.

Keywords: cardiovascular diseases, aging, disease modelling, cardioprotection

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S04. Snf1/AMPK activity is controlled by glucose transport rate and glucose phosphorylation independently by Ras/PKA pathway

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Snf1/AMPK is a highly conserved protein kinase required for energy homeostasis among all eukaryotes. In *Saccharomyces cerevisiae* it is essential for the growth on carbon sources alternative to glucose, condition in which it is active and highly phosphorylated on threonine 210 (T210) [1]. Snf1 phosphorylation is controlled by glucose catabolite repression and decrease in cells grown in high glucose condition. However, leveraging yeast strains whose glucose transport rate is decupled from glucose availability [2], we demonstrate that Snf1 phosphorylation correlates with glucose uptake rate rather than glucose concentration. Arresting the glycolytic flux at different steps, we also confirm that Snf1/AMPK dephosphorylation is controlled by glucose 6 phosphate (G6P) synthesis, coherently with in silico results supporting the direct binding of G6P with this kinase. Furthermore, Snf1/AMPK dephosphorylation is catalysed by the PP1 phosphatase Reg1-Glc7 [3], that in turn is responsive to glucose via the Ras/PKA pathway. Despite this, yeast strains compromised in the Ras/PKA pathway present a normal Snf1/AMPK response to glucose.

In conclusion, our results give new insights in the control of the activity of Snf1/AMPK and we correlate Snf1 phosphorylation to glucose uptake rate, suggesting a control mechanism independent from the activity of the Ras/PKA pathway.

Keywords: Snf1/AMPK, Glycolisis, PMI, Flux sensing

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S05. Role of Rif2 in the regulation of the checkpoint response 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 yeast Mre11-Rad50-Xrs2 complex (MRX), together with the protein Sae2, has structural and enzymatic activities to initiate DSB processing and to maintain the DSB ends tethered to each other. Furthermore, MRX activate a checkpoint response that couples DSB repair with cell cycle progression.

In budding yeast, the MRX complex is known to interact with Rif2, which is known to negatively regulate telomerase-mediated telomere elongation. We have previously shown that Rif2 modulates MRX functions at DSBs by controlling ATP hydrolysis by Rad50 [3]. It is known that the lack of Sae2 causes hypersensitivity to DSB-inducing agents and unscheduled checkpoint activation [4]. We found that the lack of Rif2 can suppress the hypersensitivity to DNA damaging agents of *sae2Δ* cells by dampening the checkpoint response. We have investigated the role of Rif2 in the modulation of the checkpoint.

Keywords: DNA damage, Genome instability, Rif2, Rad50, MRX, DNA Double-Strand Breaks, DNA Repair

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F01. New SERCA2a stimulator compounds restore impaired SERCA2a function in a rat model of diabetic cardiomyopathy

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Mechanisms that can restore cardiac relaxation (lusitropic effect) improving intracellular Ca^{2+} dynamics represent a promising therapeutic approach for cardiovascular diseases associated to diastolic dysfunction (DD). Istaroxime is a luso-inotropic agent able to stimulate SERCA2a and inhibit the Na^+/K^+-ATPase (NKA)[1-2]. New SERCA2a stimulators derived from Istaroxime have been developed in the BTBS Department thanks to a multi-disciplinary collaboration.

Overall, the aim of the project is to verify whether SERCA2a stimulation by this compounds can improve DD in streptozotocin (STZ)-treated rats thanks to a better control of intracellular Ca^{2+} handling.

In comparison to the lead compound Istaroxime, its derivatives showed much lower affinity for NKA and they stimulated SERCA2a activity at submicromolar concentration in STZ heart homogenates. Moreover, SR Ca^{2+} uptake following SR depletion became faster in the presence of each compound in STZ myocytes, thus confirming their stimulatory effect on SERCA2a. Overall, these preliminary data suggest that the novel compounds are able to restore the impaired SERCA2a activity in a pathological model of STZ-induced diabetic cardiomyopathy.

The future plan of the PhD is also understanding the role of SERCA2a stimulation by the new compounds in smooth muscle cells (i.e from the aorta and the pulmonary artery) to expand their therapeutic potential.

Keywords: SERCA, diastolic dysfunction, diabetes, Na/K pump, STZ

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F02. Charge patterning and phase separation propensity in IDPs: is there a possible interplay?

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Liquid-liquid phase separation (LLPS) underlies the formation of non-membrane bound compartments, employed to concentrate proteins and nucleic acids and further control cellular biochemistry in space and time[1,2]. Intrinsically disordered proteins (IDPs) and regions (IDRs) have been recently suggested as triggers for LLPS, due to their peculiar composition and conformational dynamism, both favouring intermolecular interactions. Electrostatic forces were proved to have a crucial role in IDR-mediated liquid demixing. Both net charge and charge patterning – *i.e.* the linear distribution of charged residues within the primary structure – seem to determine IDR propensity to phase separate, as assessed by computational[3] and experimental evidences[4,5]. Nevertheless, a unitary and comprehensive understanding of such correlation is still missing. In this respect, the connection between charge patterning and LLPS will be investigated in a human IDR, the N-terminus of topoisomerase I, which induces the relaxation of supercoiled DNA in the nucleolus. Keeping the nuclear localisation sequence unchanged, charge scrambling has been performed on the 100-residue N-terminal domain, in order to obtain synthetic constructs differing in charge distribution. Their LLPS propensity will be assessed *in vitro*, through turbidity assays and FRAP (Fluorescence Recovery After Photobleaching) measurements, and possibly *in vivo* as well.

Keywords: protein conformation, protein coacervation, linear distribution of electrostatic charges

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F03. Characterization of the influence of geometry and surface functionalization on bio-nano interaction in both physiological and pathological conditions

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One of the main hurdles to the nanoparticle (NP)-dependent targeting is the filtering action played by the liver. Resident macrophages are indeed able to uptake a high percentage of circulating NP[1]. In the last years, many studies have demonstrated that glycans may play a key-role to prolong the half-life of circulating NPs. Glycans are complex sugars that along with nucleic acids, proteins and lipids are fundamental in all living organisms. Their expression on the cell surface make them able to recognize extrinsic or intrinsic molecules, playing an important role in the immune system activation[2]. This study aims at assessing the actual influence of size, shape and glycan coating on the hepatic distribution of AuNPs after intravenous administration in healthy immune-competent mice[3]. Quantitative data achieved by inductively coupled plasma mass spectrometry and observational results by autometallography, label-free reflectance light imaging and transmission electron microscopy are combined to see the overall distribution of gold, exclude potential toxicity of the AuNPs after a short and long-term exposure, and verify the mechanisms of NP-cell interaction at ultrastructural level of resolution. This is a first step to drive the AuNPs synthesis and functionalization with different glycans as potential candidates for therapeutical purposes with liver disorders.

Keywords: biodistribution, nanoparticles, glycans, kupffer cells, nanomedicine

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F04. 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 ensure genomic stability, thus avoiding loss of genetic information and chromosome rearrangements. Eukaryotic cells can repair DSBs by two main mechanisms: non-homologous end-joining (NHEJ) or homologous recombination (HR). The initial processing of DSB ends determines which pathway is used to repair DSBs. In fact, while NHEJ requires little or no DNA end processing, HR is initiated by nucleolytic degradation of the terminated strands at both DSB ends in a process termed DNA end resection[1]. The Sae2 protein is required for the first step of DNA end resection because it stimulates the endonuclease activity of Mre11, a component of the Mre11-Rad50-Xrs2 complex that recognizes, signals and initiates repair of DSBs. The Ku70-80 heterodimer is also involved in DSB repair[2]. The Ku complex is rapidly recruited to the DSB ends and protects them from degradation caused by the resection process. The lack of Ku partially restores DNA damage resistance in *sae2Δ* cells, indicating Ku bound to the DSB ends acts as a protein block to resection[3]. To better understand the role of Ku in inhibition of DSB resection, we searched for hypomorphic and hypermorphic *ku70* mutants that show defects in DSB repair.

Keywords: Ku complex, Double-strand breaks, DNA damage response, screening, *S. cerevisiae*

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S06. Towards food traceability: discovering biomolecular technologies for complex food products

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In the agri-food sector, food frauds are increasing every year[1].The Covid-19 outbreak is getting worse the fraud issue, due to a decrease of control in the supply chain. Now more than ever is important to ensure safe and high-quality food products. DNA testing is a methodology frequently used in the food field, especially DNA barcoding methodology. DNA barcoding is a universal tool to identify species, and it currently allows for routinely market analysis [2]. However, DNA barcoding technique has some limits in the complex food products analysis. Industrial treatments could alter DNA quality of raw material, therefore this analysis could be challenging to apply. Furthermore, it can't be applied for multispecies products. Finally, this method is time-consuming and expensive so that a ready-to-use kit could be a cheaper alternative for companies. 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 [3]. We also tested metabarcoding analysis for the quality assessment of novel food insect based, analysing both plant and bacteria DNA profile [4-5]. Finally we create a mock up for a ready-to-use kit for truffle identification using the LAMP technique.

Keywords: DNA barcoding, High throughput sequencing, Isothermal amplification, Food quality, Food safety

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S07. 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 crucial to ensure genomic stability and to avoid cell death. We show that the *Saccharomyces cerevisiae* Dpb4 protein and its interacting protein complexes ISW2 [1] and Pol ε [2] are important players in the DSB response. We found that the lack of Dpb4 or the Dpb4^{A62S} mutant variant, identified in our lab, cause hypersensitivity to DSB-inducing agents and show negative interactions with the apical checkpoint kinase Tel1. We demonstrate that Dpb4 promotes nucleosome eviction around DSBs and this function is important to allow the recruitment of the MRX complex at DSBs [3] and initiation of resection of the DSB ends. Furthermore, Dpb4 plays an important role in checkpoint activation, by promoting Rad9 recruitment at DSBs that in turn activates the downstream checkpoint kinase Rad53 [5]. We suggest a model whereby Dpb4 exerts two functions at DSBs: it acts within the ISW2 complex to guarantee a histone-free context to allow resection initiation; it acts within the Pol ε complex to promote Rad9 recruitment at DSBs and checkpoint activation.

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

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S08. Tailoring non-Saccharomyces yeast cell factories to produce organic acids as products of interest

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Non- Saccharomyces yeasts are evolved to flourish in specific habitats and possess beneficial traits that have great significance in biorefineries and other production industries. These non-Saccharomyces yeasts are used in large scale production of various biochemicals by fermentation on industrial substrates. The use of renewable substrates such as lignocellulosic biomass is advantageous due to their abundance, low cost and availability. Regrettably, the pretreatments of lignocellulosic biomass, release inhibitory compounds such as organic acids that can impair the microbial performances [1] and thus it is highly important to select robust microorganisms with high tolerance to these inhibitors. The main goal of this project is to explore and compare industrial potential of two promising non-Saccharomyces yeast cell factories, *Zygosaccharomyces (para)bailii* and *Kluyveromyces marxianus* in sugar beet pulp hydrolysates for the production of organic acids. We used direct engineering method in *Z. parabailii* for studying the role of *PDR12* in tolerance towards organic acids and indirect engineering method such as adaptive laboratory evolution in *K. marxianus* to select variants tolerant to sugar beet pulp hydrolysates at low pH. Further, understanding how their desirable phenotypic traits can be linked to their genotype will lay foundation for developing these strains for the sustainable production of organic acids such as lactic acid, which has wide range of applications in various industrial sectors.

Keywords: *Zygosaccharomyces parabailii*, *Kluyveromyces marxianus*, organic acid stress, sugar beet pulp

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S09. Integrating data mining tools to explore species community patterns from HTS data

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Omics data have changed the way to study biology and biodiversity in several fields. In particular, technology advancement enables us to determine taxa composition in very different environments and, as for human microbiome, to study complex interactions in species communities [1]. Several studies revealed the complexity in inferring species associations, highlighting biases linked to sampling strategies and technical issues. In order to overcome challenges into exploring relationships between species, the aim of our work is to characterize community structures focusing on multilevel co-occurrence networks, starting from molecular data generated by HTS sequencing and developing a framework based on the association rule mining technique to reconstruct patterns from metagenomic data [2]. Considering well-known communities derived from both simulations and real experiments, our results showed that renowned biodiversity patterns are detectable. Tests revealed that taxonomy information and data composition are crucial in the data mining process and, in addition, through metadata integration and literature validation, it is possible to distinguish real biological interconnection from spurious association. Considering the huge amount of data available in repositories, a data mining strategy could be useful to collect hidden information and potentially determine undiscovered associations among organisms, paving the way to reveal functionality aspects, with the idea to integrate the method in community studies where species dynamics are still a riddle [3].

Keywords: datamining, biodiversity data, biological patterns, co-occurrences

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S10. Structural basis of flavin-based electron bifurcation: a molecular dynamics approach

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Flavin-Based Electron Bifurcation (FBEB) is a recently discovered energy coupling mechanism used by strictly anaerobic microorganisms to couple the exoergonic (high potential) reduction of a molecule with the endoergonic (low potential) reduction of another. In this process the bifurcating center is by definition a flavin-containing molecule, usually FAD, and the mechanism is catalysed by four distinct families of proteins with the best characterised being the Electron-transferring flavoprotein (Etf) [1]. Based on the crystal structure of bifurcating Etfs a model has been proposed for the electron transfer from the initial donor, NADH, to the final acceptors, ferredoxin and crotonyl-CoA. One of the step is the one-electron transfer from the bifurcating FAD molecule to another FAD in the high potential branch of the reaction, but, according to the crystal structure this transfer should be blocked by the 18Å distance between the two molecules, which is considered too long for an efficient biological electron transfer[2]. Different hypothesis has been suggested to solve this mystery, like a not-yet-seen conformational change or a hopping mechanism [2,3].

We used a structural bioinformatics approach ranging from computational chemistry to sequence alignment to help elucidate the mechanism of the electron transfer in the Etfs families.

Keywords: molecular dynamics electron bifurcation

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S11. Landscape drives changes in pollinators communities: new insights revealed by a multidisciplinary approach

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Pollinator insects are fundamental for human wellbeing, allowing plant reproduction and thus food production [1]. Unfortunately, they are facing a worrying decline of global concern [2] and this highlights the need for a deeper comprehension about the effects of human activities on pollinator communities. Here, we investigated how anthropogenic landscape features affect pollinator and plant communities in different environmental contexts. We analysed plant-pollinator communities and interactions by integrating both field observation and molecular tools, like DNA barcoding and metabarcoding, for species identification. Subsequently we combined this information with a GIS-based landscape characterization.

Analysing 264 pollinator samples collected in 26 sites in Tanzania, 490 samples from 26 sites in the district of Milan and 342 from 18 sites of Maldives islands, we found that land-use intensification is responsible for reduction in pollinators density and increased competition for resource among individuals. In a parallel study, we collected and measured several morphological traits of 350 individuals of 2 charismatic pollinators, *Bombus terrestris* and *B. pascuorum,* along an urban-rural gradient. We observed higher wing asymmetry in bumblebees from rural landscape, induced by the stressful environmental conditions they live in. Overall, we contributed with multiple approaches at increasing the knowledge about declining pollinators, also offering new insights for landscape and biodiversity management policies.

Keywords: Pollinator communities, Land use change, Biodiversity, DNA metabarcoding, Ecosystem services

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S12. Enzymatic hydrolysis of residual biomasses for carotenoids production by *Rhodosporidium toruloides*

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Biorefineries are key players in bioeconomy scenario, but their sustainability is strongly related to the origin of the feedstock. Therefore, biorefineries based on residual biomasses are increasingly of industrial interest, to overcome drawbacks of the use of edible resources. To unlock the nutrients (*i.e.* sugars) present in lignocellulosic biomasses the use of enzymes is becoming pivotal, due to their low environmental impact, available portfolio and applicability in different processes[1]. We focused our work on the exploitation of leftovers from *Camelina sativa* oil extraction, called *Camelina* meal, to produce carotenoids by fermentation of *Camelina* meal-derived sugars with the yeast *Rhodosporidium toruloides*, a natural producer of these high-value products. The saccharification step was optimized to obtain a sugar mixture then converted by *R. toruloides* into the desired product in both Separated Hydrolysis and Fermentation or Simultaneous Saccharification and Fermentation processes[2]. In addition, the loading of enzymatic cocktail was reduced to increase economic appeal to the proposed processes. Initial content of total solid was also modulated, in order to improve carotenoids productivity. A similar valorisation procedure was proposed for cinnamon residues obtained after polyphenols extraction: because of the high cellulose content, after hydrolysis the release of glucose was enough to sustain *R. toruloides* growth and carotenoids production.

Keywords: Bio-based products, Biorefinery, *Camelina* meal, Enzymatic hydrolysis, Carotenoids

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F05. Glucosinolates as nutraceuticals: process optimization and scale-up of bio-based microbial production of glucobrassicin

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Plants can produce a wide range of secondary metabolites, many of which are valuable pharmaceutical and nutraceutical compounds[1] that when in human organism can interact with the gut microbiota to produce a wide range of compounds with different effects on our health.

I focused the attention on microbial based production of glucosinolates (GLSs), which are naturally produced by members of cruciferous vegetables and possess cancer-preventive properties mainly thanks to their hydrolysis products[2]. Indeed, GLSs extraction from natural producers still poses feasibility issues at industrial scale and their chemical synthesis is challenging due to the complexity of the structures[3].

Glucobrassicin, an indolyl-methyl glucosinolate contained mainly in *Brassica* and *Raphanus* species, is the precursor of indole-3-carbinol (I3C), one of the most characterized bioactive compound showing anti-cancer properties, such as induction of apoptosis and anti-proliferative properties[4].

We started working on a recombinant strain of *Saccharomyces cerevisiae* which expresses all enzymes involved in glucobrassicin biosynthetic pathway, constructed in a previous work[5]. The first aim is to improve the titer thanks to further engineering and at the same time to develop analytical methods to evaluate the best glucobrassicin producers and the best production process. In parallel, we are working on the development of robust yeast strains that can better perform in residual biomasses.

Keywords: glucosinolates, residual biomasses, circular economy, process design, yeasts

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F06. Molecular recognition of histo-blood group antigens by human galectin-1: an NMR view

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The recognition of glycans by lectins at the surface of most cells is at the heart of important living processes. Indeed, these interactions mediate key events in biological processes such as signal transduction, cell-cell adhesion and migration. Protein-glycan interactions are also linked with several diseases, including infections, cancer or autoimmune disorders. In the last decades, several families of lectins have emerged as biomedical targets, stimulating the development of therapeutic agents against them. However, the creation of molecules able to target only a specific lectin is a formidable challenge, given the large sequence and structural similarities among the members of the same family. To achieve this goal, it is essential to dissect, at the molecular level, the crucial features of the protein-glycan recognition event. This work focuses on how human galectin-1 (*h*Gal-1) recognizes a variety of oligosaccharides, from di- (N-acetyl lactosamine) to tetra-saccharides (blood B type-II antigen). *h*Gal-1 is a β-galactoside binding lectin, which is involved in a variety of relevant biological events such as inflammatory responses, differentiation trafficking, survival of immune cells and establishment and maintenance of T-cell tolerance and homeostasis in vivo[1]. Furthermore, *h*Gal-1 has been implicated in cancer progression[2] and its overexpression in tumours has been shown to be positively correlated with a metastatic phenotype[3]. To scrutinize the binding events, a multidisciplinary approach has been adopted, combining ligand-, receptor-based and relaxation NMR experiments (STD, ^{15}N-^{1}H HSQC, CLEANEX-PM, Relaxation Dispersion) with biophysical techniques (isothermal titration microcalorimetry, ITC) and computational methods (molecular dynamics simulations, MD). Following this methodology, the atomic details of the recognition of the glycans by *h*Gal-1 have been elucidated, including the specific binding affinities as well as the thermodynamic and kinetic features of the interactions. Interestingly, the binding events display strikingly different characteristics to those reported for human galectin-3,[4] other member of the family. In particular, it is demonstrated that the entropy term favours the formation of the different complexes. Moreover, the systems display internal motions in different time scales, which are enhanced upon binding of specific ligands, supporting the existence of allostery.Overall, our results provide a comprehensive analysis of the glycan-lectin binding process, necessary to guide the design and development of galectin 1-based therapeutic tools.

Keywords: galectins, bioNMR, molecular interaction, glycans, drug design

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F07. Development of a sustainable bioprocess for Vitamin B9 Production

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Folates are essential micronutrients that act as cofactors in one-carbon transfer reactions, therefore involved in the synthesis of nucleotides and amino acids. Folate deficiency is associated with important illnesses such as anemia and cardiovascular diseases; its supplementation is therefore important for humans [1]. All the vitamins B_9 commercially available are chemically synthetized from fossil sources in the form of folic acid, which does not maximize their beneficial effects [2]. In this work, we explored complementary and alternative approaches for the production of natural folates from residual biomasses by microbial fermentation as a sustainable alternative.

The glucophilic yeast *Saccharomyces cerevisiae* was engineered in the anabolic pathways of the two main building blocks of folate, para-aminobenzoic acid and dihydropteridine, to identify the impact on the production of intra- and extracellular levels of free and poly-glutamate folates. In parallel, we performed the same measurement on alternative yeast platforms naturally able to further valorize the different sugars deriving from complex biomasses. Previous studies have demonstrated that agricolture production residues are sources of energy production and bioactive compounds that can be potentially marketable in the pharmaceutical and cosmetic industries [3]. In this study we will evaluate the possibility to produce folate by agricolture residues present in the Lombardy Region. The potential flows of resources will be identified through industrial symbiosis worktables conducted on companies in order to implement new options for their economic valorisation and new business models to increase the competitiveness of companies operating in the Lombardia Region.

Keywords: folates production, *Saccharomyces cerevisiae*, metabolic engineering, biorefinery

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F08. *Vigna unguiculata* L. Walp. seeds as source of bioactive compounds

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Vigna unguiculata L. Walp. is a plant species belonging to the Fabaceae family and better known as "cowpea". It is a species very resistant to many abiotic stressors, such as soil poorness and dry climates, and it is also a great source of micro and macro nutrients[1]. This work was aimed at characterizing and evaluating the bioactivity of cowpea extracts against colorectal cancer (CRC). Aqueous seed extracts were tested on 5 colorectal cell lines, one of which coming from healthy mucosa (CCD841) and the remaining of cancer origins (Caco-2, E705, DiFi, SW480). Extracts were found to be selectively cytotoxic against the 75% of the tumor cell lines but not on the healthy one[2]. Proven their ability to decrease the viability of many CRC cell lines, further purifications were carried out to find the principal bioactive component. Among these, Bowman-Birk protease inhibitors (BBI) were detected. At this point the gene encoding the BBI was identified and sequenced from 63 African accession of cowpea. Different isoforms of this gene were identified, corresponding to different modifications in the amino acidic sequence. Further investigation will be carried out to evaluate if the abovementioned isoforms show different bioactivity levels and if any evolutionary process on the selected gene are occurring.

Keywords: *Vigna unguiculata*, Plant extract, Colorectal cancer, Bowman-Birk Protease Inhibitor

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S13. Membrane engineering to improve *Saccharomyces cerevisiae* robustness towards organic acids

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During bio based industrial processes yeasts are challenged to perform in adverse conditions, such as low pH, high temperatures and in the presence of inhibitors derived from biomass. Organic acids (OAs) play a unique role in these processes since they are often released from biomass treatments and, when in the fermentative broth they can compromise the cell metabolism and cause growth arrest[1]. OAs can also be products of interest obtained from microbial factories, with applications in several industrial sectors. Therefore, controlling the cellular inward/outward flux of OAs can improve fermentation performances.

Plasma membrane plays a pivotal role in this process, acting as a selective gate and changing its composition in response to the presence of OAs[2]. Thus, its engineering has been envisaged as a strategy to increase yeast performance. We are evoking a membrane rewiring focusing on the modulation of the transcription factor *ECM*22, involved in the regulation of ergosterol biosynthesis[3].

Here we show how changes in the cellular content of ergosterol can have a positive or negative effect on yeast robustness depending on the molecular structure of the OAs. Furthermore, these effects can vary according to other parameters relevant for industrial processes, such as temperature.

Keywords: plasma membrane, stress, cell factory, robustness

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

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The human gut is inhabited by communities of microorganisms, which composition evolved to exploit beneficial tasks for the host, meanwhile thrives in sites providing a nutrient-filled habitat. Therefore, preserving gut microbiota through the administration of prebiotics, probiotics, or their combination known as synbiotic, represents a new strategy to improve human health. Hence, during the first year of my PhD project, a synbiotic formulation was developed. *Lactobacillus plantarum* LP, *Lactobacillus acidophilus* LA, and *Bifidobacterium animalis* subsp. *lactis* BL[1-2-3] were chosen as the most promising for an *in vivo* intervention study, combined with mixtures of fructooligosaccharides with a degree of polymerization up to 20[4], because they were highly fermented by the identified probiotic strains. In the second year, the modulation of the intestinal microbiota composition and the stimulation of the immune system by the synbiotic were assessed on healthy elderly subjects. The microbiota of the subjects treated with the synbiotic formulation showed the highest biodiversity, linked to the variation rate of seven beneficial taxa: *Akkermansia*, *Bifidobacterium*, *Blautia*, *Faecalibacterium*, *Prevotella*, *Roseburia*, and *Ruminococcus.* Furthermore, there was a correlation between the effects on the microbiota and the considered clinical biomarkers (faecal β-defensin and calprotectin; salivary IgA and total antioxidant capacity). Notably, the levels of β-defensin 2 increased, whereas calprotectin amounts decreased [5].

Keywords: prebiotics, probiotics, gut microbiota

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S15. Design and synthesis of new bio-functional materials for 3D models and tissue engineering applications

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Tissue engineering is a rapidly growing discipline that seeks to develop functional structures able to mimic the tissue environment to restore, replace, or enhance biological structure and functions that have been lost due to injuries or pathologies. The strategy is to develop synthetic extracellular matrices to build up 3D living structures with embedded cell populations, to induce functional, structural and mechanical properties equal to the tissue that we seek to reproduce [1,2]. The leading actors are cells, the material scaffolds and the regulatory signals, as growth factors, peptide motifs or glycidic cues, involved in the orchestration of tissue physiology, morphology and mechanical properties. For this aim, 3D printing is capable of producing scaffolds fabricated to organ-specific requirements [3].

The challenge of the project is to engineer human body tissue as 3D scaffolds in order to emulate patterns of biological structure, using functionalized biopolymers combinations [4]. Therefore, it is necessary to understand the ECM physical, chemical, and biological functions in physiological or pathological conditions and use these kinds of information to fabricate 3D scaffolds. Here, in this project, the design of combined polymers to mimic tissue structures and functionalities have been employed as a scaffold or bioprintable inks for the 3D control of cell-cell and cell-ECM interplay.

Keywords: Bioprinting, Hybrid Hydrogels, Scaffolds, Diels-Alder click Chemistry, Polymers functionalization

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Theory enhancing Projects. Title: Dissecting the link between pulmonary stromal changes and lung cancer progression for biomarkers discovery and therapeutic intervention.

S16. Stepping towards the future of tissue engineering: *in-silico* modeling of cell-biomaterial interactions

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The development of effective tissue engineering treatments lay on the deep understanding and control of the biomechanical interactions between cells and biomaterials. The recent advances in the field of computational chemistry and NMR spectroscopy, shed new light on the structure-properties relationship of nature-inspired materials, such as self-assembling peptides (SAPs).[1][2][3] More in details, atomistic and MARTINI coarse-grained molecular dynamics (CG-MD) simulations have proven to be suitable for the elucidation of SAPs biomaterials organization.[2][3][4] Indeed, MARTINI CG-MD simulations, combined with dedicated software, such as home-made Morphoscanner, have allowed to investigate conformational aggregation patterns of SAPs.[1][2] Furthermore, steered MD simulations have been used to investigate the mechanical properties and thermodynamic stability of SAPs fibrils.[5][6] Such achievements open up new dimensions in the field of biomateriomics, allowing to elucidate the complex interplay between cell membranes and artificial bio-nanostructures.[5][7] In addition, the combination of the above mentioned methods will unveil the effects of the neo-glycosylation on SAPs organizations and interactions with neural membranes.

Keywords: Biomaterials, peptides, neural membrane, aquaporin, biomechanics

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S17. Anti-inflammatory and regenerative potential of extracellular vesicles for osteochondral repair

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Osteoarthritis (OA) is characterized by the lack of early diagnostic biomarkers, effective therapeutic approaches and screening platforms to evaluate them[1]. To validate in terms of chondral differentiation the OA joint-on-a-chip model developed in the first year, I assessed gene expression with droplet digital PCR, developing an ad-hoc protocol to collect cells from the device and to extract RNA.

Within this model, the role of extracellular vesicles (EVs) as innovative biomarkers and OA treatment[2,3] will be evaluated. To assess the potential of synovial fluid-EVs as OA biomarker, I'm isolating and separating the different EVs subpopulations from OA patient synovial fluid. To this end, I'm employing different techniques: differential centrifugation, size-exclusion chromatography and asymmetrical flow-field-flow fractionation. Once separated, I characterize each subpopulation by evaluating both general characteristics (by nanoparticle tracking analysis, western blot, total protein quantification) and single-vesicle parameters, by atomic force microscopy.

During the next year, I will perform proteomic analysis of the cargo of EVs belonging to each subpopulation, focusing on the pathways involved in OA: inflammation, metabolic imbalance and extracellular matrix remodeling. Finally, I will investigate possible different roles of EVs subpopulations by injecting and tracing them in our microfluidic model, assessing their effect on articular cells.

Keywords: Osteoarthritis, Extracellular vesicles, joint-on-chip, microfluidic

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S18. Development of innovative Q-LAMP assay for direct detection and amplification of flu A/B and RSV genomes

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Despite decades of surveillance and interventions, influenza viruses (especially types A and B strains[1]) still represent a public health concern[2]. Respiratory syncytial virus (RSV) also infects the respiratory system causing an influenza-like illness in infants, toddlers and high-risk adults[3].
Despite accuracy of diagnosis based on clinical presentation, fast and specific tests are required to confirm virus-specific infection[4].
PCR-based assays are commonly used[5], 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 and RSV, with similar efficiency, speed and sensitivity. The thermal protocol has been optimized introducing a sample-processing step ensuring detection of not-extracted viruses. Finally, reagents have been balanced and optimized to obtain results in less than 60 minutes.

Keywords: Influenza, RSV, Q-LAMP, Direct detection, Molecular assay

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S19. Microorganisms and hydrocarbon pollution: the strategy of biosurfactant producers

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The remediation of polluted soils will be one of the major EU challenges in the coming years. Indeed, there are more than 650,000 contaminated sites registered in national and regional inventories, and more than 65,500 sites have been already remediated or are under aftercare measures, with an increment of more than 8,500 new remediated sites in the last 5 years (JRC 2019)[1]. Besides having a relevant impact on society and environment, polluted sites are an important source of microorganisms with industrial application potential.

While working at the bioremediation of the Fidenza Site of National Interest (SIN), we have isolated severals microorganisms able to degrade polyaromatic hydrocarbons (PHA) while at the same time being important producers of biosurfactants. Biosurfactants are used by microorganisms as a strategy for a better degradation of hydrophobic carbon sources. Biosurfactants are also an eco-friendly alternative to chemical synthesis surfactants potentially applicable to many industrial products and processes. In this work we describe four biosurfactant producer microorganisms which have been successfully used to mimic pilot scale industrial production in view of the use of their products in the cosmetic sector.

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S20. Unconventional purification strategies of reagents for immunodiagnostic kits

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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 third year of Phd we worked on purification process of C33 biotinylated obtained from an innovative site-specific conjugation method: the Split-intein system[5].

Keywords: Immunodiagnostic, Alternative purification processes, Site-specific biotinylation, Chromatography, Recombinant proteins

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S21. Unraveling the contribution of melanoidins to the anti-inflammatory activity of roasted coffee extracts

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Coffee beans have been used for centuries for preparing a non-alcoholic drink that has become one of the most widely consumed beverages on the planet[1]. Due to its health benefits, coffee is attracting attention of researchers[2][3][4]. Inflammatory bowel disease (IBD) represents a group of intestinal disorders that cause prolonged inflammation of the digestive tract[5]. This study aimed to investigate the capability of coffee extracts (CE) to prevent inflammation in the gastro-intestinal tract. To examine the in vitro preventive anti-inflammatory activity of green and roasted coffee extracts, we performed cell-based assays, employing macrophage and intestinal epithelium cell models, measuring pro-inflammatory cytokines released following an inflammatory stimulus, in presence or absence of extract pre-treatment. Moreover, we investigate the contribution of the major components of the two extracts tested, respectively chlorogenic acid (5-CQA) and melanoidins, in order to find the molecular entities responsible for the preventive anti-inflammatory effect that we have observed. Results indicate that both 5-CQA and melanoidins contribute to the overall anti-inflammatory capability of green and roasted coffee extract in macrophages and enterocytes (TNF-α and IL-8, respectively). These findings raising the awareness of coffee consumers and not consumers for the high intake of 5-CQA and melanoidins through coffee and for their potential beneficial health effects in preventing bowel inflammation.

Keywords: inflammation, prevention, phytochemicals, coffee extracts, roasting, melanoidins

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S22. Mammary carcinoma spheroids for the study of cancer metabolic plasticity and stemness properties

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Treatment failure in breast cancer is often caused by acquired resistance and spread of metastasis[1,2]. Metabolic rewiring represents one of the mechanisms through which cancer cells could become more aggressive[3,4]. In particular, a sub-population composing the tumor mass named as 'Cancer stem cells' (CSC) seems to adapt its metabolism to microenvironmental changes by shifting energy production from one pathway to another[5]. Therefore, metabolic alterations can potentially be exploited for new therapeutic intervention. Moreover, three-dimensional (3D) models may represent a valid compromise between monolayer cultures and *in vivo* models for studying the complexity and heterogeneity of mammary carcinoma sub-populations.

For this study we generated spheroids (3D cultures) from the following breast cancer cell lines: SUM159PT (triple-negative, primary tumor), MDAMB231 (triple-negative, metastasis), MCF7 (hormone receptors positive, metastasis). Seahorse technology and flow cytometry have been used to perform mitochondrial analysis, cell cycle evaluation and the expression of various markers of differentiation on the spheroids produced from each cell line and, when possible, compared to the monolayer counterpart. Spheroids showed a higher mitochondrial functionality correlated with a loss of markers of differentiation.

In the future experiments, high resolution imaging will be employed to evaluate further morphological and metabolic features of breast cancer 3D cultures and to study their drug response.

Keywords: 3D models, spheroids, Breast cancer, metabolism, cancer stem cells

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S23. Evaluating cadmium effect on LUHMES cell line

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Cadmium (Cd) is a widespread toxic pollutant, released into the environment mainly by anthropogenic activities. Human exposure can occur through different sources: once absorbed it accumulates throughout a lifetime (biological half-life of 20-30 years) exerting toxic effects on different target organs. Cd exposure has been related to impaired functions of the nervous system and to neurodegenerative diseases[1]. This heavy metal may enter the brain by increasing blood brain barrier permeability or through the olfactory nerves, exerting its toxicity in several ways, such as interfering with essential metal ions homeostasis or depleting cell's antioxidant defence systems[2,3].
In this work the LUHMES (Lund human mesencephalic) cell line has been used as neuronal model system for the evaluation of Cd toxicity. Metal administration caused a dose-dependent reduction in cell viability, and in GSH and ATP content. Moreover Cd treatment induced the activation of Nrf2 and ATF4 and its adversity could be rescued by GSH addition.
Finally Cd effect on cell viability was investigated in a co-culture model of LUHMES and BV2 (murine microglia cell line) and in the presence of astrocyte-conditioned medium, showing not only a protective effect by glia, but also the importance of more complex cell lines models.

Keywords: LUHMES, cadmium, neurons, co-culture, viability

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F09. Role of SP-D protein during SARS-CoV-2 infection for the development of a therapeutic treatment

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The pulmonary surfactant is formed by lipids and proteins called Surfactant Protein (SP-A, B, C, D).[1] SP-D is an hydrophilic protein characterized by an immunomodulatory function removing the foreign matters (*e.g.* bacteria or viruses) after the binding between its Carbohydrates Region Domain (CRD) and the glycoproteins of the pathogens.[2] The basic structure of SP-D is the trimeric one from which the oligomerization in multimeric structures develops.[3] Considering that in literature the SP-D is described as the key player of the active regulation of immune response in the pulmonary environment,[4] this project aims to investigate the role of this protein during SARS-CoV-2 infection.
We reasoned to synthesize liposomes composed by lipids of pulmonary surfactant decorated with SP-D to mimic its multimeric structures. This fully biocompatible nanoparticles are expected to promote the clusterization of viral particles and the prevention of the alveolar epithelial cells' infection. After characterization, both SP-D and SP-D-conjugated liposomes were tested *in vitro* to confirm their ability to interact with bacteria cells causing their agglutination. The next step will be the evaluation of SP-D-conjugated liposomes antiviral activity against the SARS-CoV-2 infection.

Keywords: SARS-CoV-2, protein, SP-D, bacteria, liposome

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F10. DALIS in aging, inflammation and neurodegeneration

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Neurodegenerative diseases are debilitating pathologies with a common underlying pathogenic mechanism, involving incorrect protein folding processes and protein aggregation. In this project, we will focus our attention on the study of dendritic cell aggresome-like induced structures (DALIS), a type of membraneless protein aggregates composed by defective ribosomal products (DRiPs) typical of dendritic cells[1]. In the first instance, a wide data mining was conducted with the aim of selecting different biological models, including mammalian dendritic cells, neurons and yeast cells, to exhaustively evaluate the role of protein aggregates in neurodegenerative diseases. In particular, *Saccharomyces cerevisiae* was identified as the most suitable starting point, being a well-established and powerful model organism to study metazoan proteins associated to such diseases[2]. Databases were thoroughly searched in order to determine which stress conditions could lead to the formation of membraneless protein aggregates in yeast cells. Heat shock temperatures, amino acid and glucose starvation were addressed as the main sources of stress able to induce such aggregation[3]. Moreover, it was shown that the formation of DALIS is enhanced by puromycin administration[4]. Since yeast is naturally resistant to puromycin[5], a puromycin-sensitive triple mutant (EPP), lacking the ergosterol 6 (erg6), pleiotropic drug resistance 1 (pdr1) and 3 (pdr3) genes, of the BY4741 strain was obtained[6]. Therefore, wild type and EPP yeast cells were subjected to these stress conditions and analyzed by western blot analysis and confocal microscopy.

Keywords: DALIS, *Saccharomyces cerevisiae*, membraneless protein aggregates

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F11. Design and development of engineered biomimetic nanoparticles for the investigation of cross-talk between cancer associated fibroblasts (CAFs) and cancer cells.

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest malignancies[1]. Both in resectable and nonresectable PDAC, chemotherapy remains the preferred treatment[2]. However, its failure is mostly attributable to a desmoplastic stroma that hampers efficient drug delivery[3]. Tumor microenvironment (TME), an interacting network of malignant and non-malignant cells within a complex extracellular matrix, plays key role affecting tumor progression and resistance[4,5]. Cancer-associated fibroblasts (CAFs) represent a pivotal player in TME modulation, promoting a bidirectional crosstalk with neighboring tumor cells[6,7]. Taking advantage of CAFs crosstalk with surrounding cancer cells and easy access via blood vessels, we propose an innovative therapeutic strategy consisting in reprogramming CAFs by means of gene therapy to become an active source of tumor-specific proapoptotic cytokine that will be released in direct proximity of tumor mass. Cancer cell membrane-derived biomimetic nanoparticles (CCMNPs) will be used as CAF-targeted vectors thanks to their natural tropism towards TME[8]. Plasmids encoding TNF-related apoptosis-inducing-ligand (TRAIL) will be specifically delivered to induce CAF production of excretable forms of soluble or exosome-associated TRAIL, mimicking the paracrine crosstalk between cancer cells and CAFs. The excreted TRAIL, in turn, will target the nearby tumor cells and exert its cytotoxic activity, resulting in cancer removal.

Keywords: biomimetic nanoparticles, tumor microenvironment, cross-talk, cancer associated fibroblasts

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F12. Engineered extracellular matrix biomimetics for advanced 3D *in vitro* models

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Extracellular matrix (ECM) is a complex three-dimensional network composed of fibrous proteins, glycoproteins, and polysaccharides representing the noncellular part of tissues[1]. ECM features are multifarious, and the structural role is enriched by a large plethora of functions, including cell adhesion, intercellular interactions and cell fate control. The ECM composition varies across tissues and is involved in the maintenance of homeostasis and cell differentiation during tissue morphogenesis[2]. Therefore, functional artificial ECMs applied to 3D cell culture represent a powerful translational model for drug discovery and tissue engineering, displaying multiple advantages over the classical 2D model[3]. Hydrogels are versatile platforms for such purpose since provide a bioactive and bioresponsive microenvironment for cell adhesion, differentiation, and proliferation[4]. Tuning their biochemical composition and physicochemical properties, it is possible to develop hydrogels suitable for different applications. The combination of hydrogels with living cells can be referred to as bio-inks, which is employed in 3D bioprinting, the state-of-the-art technology for the biofabrication of multicellular tissues mimicking in size, shape and geometrical complexity the native counterpart[5]. Here in this project, different combinations of natural and synthetic polymers have been designed, synthesized, and characterized. Finally, they have been preliminary tested for tissue engineering and 3D in vitro applications.

Keywords: tissue engineering, biomaterials, 3D bioprinting, hydrogel, ECM, glioblastoma.

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F13. Glutamate as a potential substrate for trehalose production in yeast

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Trehalose, a disaccharide involved in cellular fundamental processes, is also considered a valuable commodity for chemical, pharmaceutical and food industries[1]. State-of-the-art production methods involve cellular/enzymatic biotrasformation from dextrins and oligosaccharides chains which lead to high yields but require a complex purification step from a mixture of several sugars[2]. Trehalose produced from yeast cells is very pure, but cell lysis and purification steps are required. Therefore, an innovative method to produce trehalose by yeast redirecting excretion to the culture medium was investigated. When triggered by a singular growth condition, a specific *Saccharomyces cerevisiae* strain is induced to use glutamic acid as the only nitrogen and carbon source. This strain showed a transient capability to redirect fluxes from glutamic acid towards the production of reserve carbohydrates. We found that trehalose reaches concentrations close to those reported in production but avoiding typical carbon or nitrogen starvation steps. From these starting points, I worked to further increase trehalose titre, to promote trehalose excretion and to optimize the use of glutamate. Adaptive laboratories evolution, gene overexpression and deletion were performed also using different yeast strains. Finally, a kinetic model was developed in order to describe the essential reactions involved in glutamate assimilation and to predict different feeding profiles protocols which could allow to maximize trehalose production in a bioreactor.

Keywords: trehalose, *S. cerevisiae*, glutamic acid, regulation of metabolism, trehalose synthase

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T01. Human herpesvirus genetic diversity and host adaptation

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Herpesviruses belong to a diverse family (*Herpesviridae*, order *Herpesvirales*) of enveloped, double-stranded DNA viruses that infect a wide range of animals and often establish life-long infections. In analogy to observations in other mammalian and non-mammalian hosts, herpesvirus infection is remarkably species-specific in primates, at least in natural settings. In line with these observations, the phylogenetic relationships among herpesviruses very often mirror those among their hosts, indicating that viral lineages frequently arose through co-speciation with host lineages. As a consequence, it is generally assumed that human herpesviruses *originated in Africa and dispersed during early human migration events. However, our recent data indicated that this is not necessarily the case. For instance, extant VZV strains did not disperse from Africa, but from Europe, and HSV-2 circulating strains have relatively recent ancestry.*
Some human herpesviruses such as HCMV display a remarkable species-specificity even in vitro - i.e., in the absence of the host adaptive immune response. This clearly implies that HCMV must have adapted to efficiently complete its infectious cycle in human cells. In line with this view, analyses of primate cytomegalovirus genomes indicated that core viral genes were targeted by positive selection during HCMV speciation. Some adaptive variants, though, were found to decrease replication in human cells, suggesting HCMV evolution towards viral temperance. Compared to HCMV, herpes simplex viruses have less strict species-specificity and cross-species transmission events have been described (often with very serious consequences for the non-natural host). In line with this view, genes involved in immune evasion were the major targets of selection during the adaptation of herpes simplex viruses to hominins. However, we have recently found that the evolution of ICP47 in HSV-1/HSV-2 led to the loss of an immunosuppressive effect, suggesting that simplexviruses finely tune the balance between immunosuppressive and immunostimulatory pathways to promote successful co-exisence with their primate hosts. In summary, evolutionary analyses of human herpesvirus extant diversity can provide relevant information on the dispersal and selective patterns of these extremely prevalent human pathogens

T02.THE DARWINIAN LESSON OF COVID19

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We should look at Covid19 through evolutionary lenses. The Darwinian lesson of the coronavirus is clear: we are vulnerable, we are connected to the rest of nature, the destruction of the environment turns against our health. Spillover pandemics don't come out of nowhere. They have specific ecological causes and human activities that increase their likelihood: deforestation, poaching, illegal trade in endangered animal species, etc. Viruses are biological entities much older than us and have basic and very effective evolutionary strategies. Moreover, eight billions humans have become perfect hosts for viruses, which travel with us by plane. On the other hand, viruses have four formidable opponents: scientific research (vaccines; Global Virome Project; atlas of pathogens); hygiene; social progress; and environmental protection. To defeat such an enemy, we have to put ourselves in its point of view, and understand its logic, an evolutionary logic.

T03. Bacteria feeding plants helps life on the planet

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Indole-3-acetic acid (IAA) is the main auxin acting as a phytohormone in many plant developmental processes. The ability to synthesize IAA is widely associated with plant growth-promoting rhizobacteria (PGPR) as long as for pathogenic bacteria. Rhizobia are symbiotic bacteria able to penetrate legumes roots, trigger the formation of root nodules where atmospheric nitrogen is reduced into ammonia or amino acids by the complex nitrogenase enzyme. This process is the main source on nitrogen available for any living organism. We introduced in a rhizobia strain an additional pathway for IAA biosynthesis leading to the Ensifer meliloti strain RD64 and used it to inoculate Medicago sativa plants. The overproduction of bacterial IAA inside root nodules improves the activity of the nitrogen-fixing apparatus and the photosynthetic function providing the energy required for breaking the triple bond of the dinitrogen molecule. Furthermore, the RD64-nodulated plants showed a biomass increase over time, with the highest increment (more than 60%) being reached at six weeks after infection. The action of IAA on the nitrogen fixation apparatus is not exclusive for legumes and endophytic bacteria colonizing cereals as rice show similar behavior. We are investigating a possible mechanism of action of IAA.

T04. GENOME (sequencing) EVOLUTION

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Since the completion of the human genome project in 2001, extraordinary progress has been made in genome sequencing technologies, which has brought the cost of sequencing a human genome down to around US$1,000, thus enabling the use of sequencing as a clinical tool.

Although exciting, these advancements are not without limitations. As new technologies emerge, existing problems are exacerbated or new problems arise. Current “Next Generation Sequencing” (NGS) platforms provide vast quantities of data, but the associated error rates (~0.1–15%) are higher and the read lengths generally shorter than those of traditional Sanger sequencing platforms, requiring careful examination of the results, particularly for variant discovery and clinical applications. Long-read sequencing technologies are becoming available, but their error rate and cost per gigabase are much higher, thus limiting their widespread adoption.

The fast and low-cost sequencing platforms now available are providing physicians with the tools needed to translate genomic information into clinically actionable results. However, there are many limitations in the current genomic sequencing approaches that we must understand and keep into account when performing analyses, especially in the clinical settings. Understanding the current limitations of genomic technologies and applying these technologies and their methods to tackle the appropriate question is therefore a fundamental aspect of precision medicine.

So, there are many technologies now available for genomic analyses, but which is the right one for your application? In order to answer to this question, I’ll give an overview of current and emerging genomic technologies focussing on their pros and cons when adopted in a research environment and in a clinical setting.