Dottorato in Biotecnologie
Università degli Studi di Perugia

Winter School on Biotechnology

22-26 January 2018
Teaching Center of the School of Medicine

The School is made by lectures and open discussions tailored for a broad audience of PhD students and young researchers from university, research centers and industries who are entering the fascinating, multi-disciplinary field of Biotechnology. This year, the School addresses emerging methods and technologies in the field of ***genomics and beyond*** with invited lectures, seminars and posters by the participants.

Pre-registration is mandatory, up to a **maximum of 50 students**
info: dottorato.biotecnologie@unipg.it

Invited Lectures (preliminary list):

FABIO BISCARINI, Life Sciences Dept.- University of Modena and Reggio Emilia
Organic Bioelectronics: from fundamentals to applications to biosensors and implantable devices

PIERSANDRO COCCONCELLI, Università Cattolica del Sacro Cuore, Piacenza
Microbial Metagenomics

ANNA MIGLIAZZA, Head, External Reseach Nerviano Medical Sciences
New drug R&D: How we translate biology into novel candidate therapies

VALERIO ORLANDO, Head of KAUST Environmental Epigenetics Res.Progr., King Abdullah Univ. of Sci. & Tech
Above the DNA: the role of the Epigenome in cell identity, plasticity and reprogramming

FRANCESCO PAVONE, UniFi and European Laboratory for Non-Linear Spectroscopy (LENS)
Morpho-chemistry of tissues by optical imaging

DOMINIQUE SOLDATI-FAVRE, Dep. of Microbiology and Molecular Medicine, University of Geneva
Genomic Manipulation in Apicomplexan Parasites

Dottorato in Biotecnologie
Università degli Studi di Perugia

Winter School on Biotechnology 2018

PROGRAMMA

Monday, January 22, 2018
Chair: Sabata Martino

■ LESSONS ■ PRESENTATIONS

9:00 – 9:30		
9:30 – 11:00	**Valerio Orlando**	Above the DNA: the role of the Epigenome in cell identity, plasticity and reprogramming
11:00 – 11:30		COFFEE BREAK
11:30 – 13:00	**Francesco Pavone**	Morpho-chemistry of tissues by optical imaging
13:00 – 14:00		LUNCH
14:00 – 15:30	**Fabio Biscarini**	Organic Bioelectronics: from fundamentals to applications to biosensors and implantable devices
15:30 – 16:00	**Beatrice Gironi**	Low temperature behavior of model lipid membranes, hydration and more
16:00 – 16:30	**Federica Piro**	Dissection of the TORC pathway in Toxoplasma gondii
16:30 – 17:00	**Chiara Antonini**	Analysis of dynamic networks in proteomics data of NSCLC cell lines using a new pipeline based on machine learning tools

Tuesday, January 23, 2018
Chair: Stefano Bruscoli

9:00 – 10:30	**Anna Migliazza**	New drug R&D: How we translate biology into novel candidate therapies
10:30 – 11:00		COFFEE BREAK
11:00 – 12:00	**Efisio Puxeddu**	Use of NanoString technology to define the immune profile of thyroid carcinoma
12:00 – 13:00	**Olga Britanova**	Application of the molecular barcoding in immune repertoire profiling
13:00 – 14:00		LUNCH
14:00 – 15:00	**Gennaro De Vita**	Features of antibodies for life science research. Applications in proteomic field
15:00 – 15:30	**Sara Flamini**	Glucocorticoid and B lymphocytes function: role of glucocorticoid-induced leucine zipper (GILZ)
15:30 – 16:00	**Annita Montepeloso**	Improving the ability of hematopoietic stem and progenitor cells to generate microglia-like progeny upon transplantation
16:00 – 16:30	**Samuele Sabbatini**	Effect of probiotic on Gardnella vaginalis aggregation and adhesion to epithelial cells

16:30 – 17:00	**Krizia Sagini**	The effect of drug induced phospholipidosis on extracellular vesicles release

Wednesday, January 24, 2018
Chair: Loredana Latterini

9:00 – 10:30	**Massimo Vassalli**	Active mechanosensing in living cells: the emerging role of piezo channels in human patho-physiology
10:30 – 11:00		COFFEE BREAK
11:00 – 12:00	**Tamara Posati**	Natural polymers as advanced materials for bio-medical devices
12:00 – 13:00	**Ermanno Federici**	Metagenomic profiling of microbial communities in extreme environments
13:00 – 14:00		LUNCH
14:00 – 15:00	**Stefano Zancan**	Clinical Development: Enabling Steps to enter into the Clinic
15:00 – 15:30	**Francesco D'Angelo Francesco Martino Carpi**	Informatics solution for personalized medicine approaches
15:30 – 16:00	**DIATEC**	
16:00 – 16:30	**Chiara Argentati**	Stem cell-biomaterial interaction steers stem cells toward a selected specification lineage
16:30 – 17:00	**Eleonora Calzoni**	Proteases immobilization on innovative material for the degradation of agribusiness biomasses in white biotechnology applications

Thursday, January 25, 2018
Chair: Efisio Puxeddu

9:00 – 10:30	**Dominique Soldati-Favre**	Genomic Manipulation in Apicomplexan Parasites
10:30 – 11:00		COFFEE BREAK
11:00 – 12:00	**Edoardo Puglisi**	Understanding the structure and functions of microbial communities in the big-data and post-genomic era
12:00 – 13:00	**Giuseppe Bellisola**	VibroSpectrOmics: just a Star Treck technology?
13:00 – 14:00		LUNCH
14:00 – 15:00	**Duccio Cavalieri**	Role of the human Mycobiome in health and disease
15:00 – 15:30	**Casagrande Pierantoni Debora**	Candida biofilm: a multidisciplinary approach to gain insight on its structure and growth
15:30 – 16:00	**Nicole Nucci**	Involvement of the Aryl hydrocarbon receptor (AhR) in thyroid cell transformation
16:00 – 17:00	**POSTER SESSION**	

Friday, January 26, 2018.
Chair: Gianluigi Cardinali

17:30 – 19:30 **Sala della Galleria Nazionale dell'Umbria** – C.so Vannucci
Tavola rotonda aperta al pubblico sul tema:

Biotecnologie e Società
La Rivoluzione dei Big Data per lo scienziato e per tutti noi

Intervengono:
Prof. Duccio Cavalieri – Università di Firenze
Prof.sa Flavia Marcacci – Università Lateranense
Mons. Tomasz Trafny PhD – Segretario Esecutivo Fondazione STOQ Pontificio Consiglio della Cultura

Biotecnologie oggi
L'incontro di orientamento con studenti e società, sarà organizzato in collaborazione con la Fondazione ITS – Umbria ed il dottorato di ricerca di Biotecnologie.
La data dell'incontro sarà comunicata durante la Winter School 2018.

INDEX OF PRESENTATIONS

Oral Presentation

Posters

Abstracts

O1/P1. Low-temperature behaviour of model lipid membranes:hydration and more

Beatrice Gironi [a], Marco Paolantoni [a], Assunta Morresi [a], Paolo Foggi [a, b], Paola Sassi [a, c]
[a] Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy
[b] European Laboratory for Non Linear Spectroscopy (LENS), Università di Firenze, via Nello Carrara 1, 50019 Sesto Fiorentino, Florence, Italy
[c] Centro di Eccellenza sui Materiali Innovativi Nanostrutturati (CEMIN), Università di Perugia, Via Elce di Sotto 8, 06123Perugia, Italy

The properties of lipid membranes at low temperatures are important for a number of biomedical and biotechnological applications and the success of these applications depends on understanding the effects of temperature changes on intermolecular lipid-lipid and lipid-water interactions. Fourier Transform Infrared Spectroscopy (FTIR) is a powerful and label free technique largely used to investigate the structure of lipid membranes since we can obtain information about conformation and dynamics of all membrane regions at the same time[1]. Here we use FTIR spectroscopy to study different lipid suspensions in water/DMSO solutions in the -60÷30 °C range; the choice of DMSO as a cosolvent is justified since it is one of the most used cryoprotective agents of cellular systems. In the present work we analyse the effects of the solvent composition on the structural and thermotropic properties of liposomes composed of an unsaturated lipid and cholesterol since they are a suitable model of plasmatic membrane[2]. To this extent, we compare the properties of lipid vesicles suspended in water/DMSO solution at 0.0 and 0.1 DMSO mole fraction.

1. R.N.A.H. Lewis, R.N. McElhaney, Biochim. et Biophys. Acta 1828 (2013) 2347 – 2358
2. K. Simons, W.L. Vaz, Annu. Rev. Biophys. Biomol. Struct., 33 (2004) 269–295

O2/P2. Dissection of the TORC pathway in Toxoplasma gondii

Manlio Di Cristina [a], Federica Piro [a]
[a]University of Perugia, Department of Chemistry, Biology and Biotechnology, Building B, Via del Giochetto, 06122 Perugia, Italy

Autophagy is a mechanism able to produce nutrients when a cell is in starvation in order to maintain it viable. Toxoplasma gondii exploits this mechanism in its life cycle and we want to understand how autophagy is important in the survival of this parasite. To better understand, we decided to study which are the most important regulator proteins in T. gondii that are involved in the regulation of autophagy.
Thanks to bioinformatics analysis we selected some proteins that may play key roles in autophagy regulation, i.e. TGME49_227430, TGME49_227570 e TGME49_227580. These proteins have been predicted as lysosomal amino acid transporters and sensors similarly to SLC38A9, Rag-a Rag-c and LST8 proteins that have a role in regulation of TORC1 complex in humans[1].
Our aim is to characterize these proteins in terms of role and localization in different stages of the T. gondii life cycle and identify possible therapeutic targets to eradicate this parasite infection[2].

1. Rebsamen M., Superti-Furga G. (2016). SLC38A9: a lysosomal amino acid transporter at the core of the amino acid-sensing machinery that controls mTORC1. Autophagy 12(6): 1061-1062.
2. Yao Y., Jones E., Inoki K. (2017). Lysosomal Regulation of mTORC1 by Amino Acids in Mammalian Cells. Biomolecules. 7(3): 51.

O3/P3. Analysis of dynamic networks in proteomics data of NSCLC cell lines using a new pipeline based on machine learning tools.

Chiara Antonini [a], Lorenzo Tomassoni [a], Elisa Baldelli [b], Vienna Ludovini [c], Sara Baglivo [c], Mariaelena Pierobon [b], Emanuel F Petricoin [b], Lucio Crinò [d], Paolo Valigi [a], Fortunato Bianconi [e]

[a] University of Perugia, Perugia, Italy; [b] George Mason University, Manassas, VA; [c] Azienda Ospedaliera di Perugia, Perugia, Italy; [d] Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy; [e] Independent researcher, Montefalco, Italy.

Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer. The aim of this study was to develop a systems biology approach to understand complex interactions in NSCLC and predict response to target treatments. We combined proteomic data with machine learning to analyze eight NSCLC cell lines (CL) (5 KRAS MUT and 3 KRAS WT). CL were subjected to reverse phase protein microarray (RPPA) to measure 183 proteins treated with Selumetinib (SE), at 6 time points. We applied Support Vector Machine (SVM) to discover the most divergent proteins both between control and treated samples and between KRAS MUT and WT CLs. To build the interaction networks from these subsets, we used two Cytoscape plugins. The algorithm revealed that SE was mainly effective in a short time, since the network at 1 hour contained 25 proteins and 76 interactions, while at 24 hours only 4 proteins. The output network included not only the SE target (MEK), but also other pathways, such as NGFR, mTOR and PI3K-Akt. Moreover, in the first hour, MEK and ERK were the main relevant proteins in WT samples, while in MUT samples a complex network of 70 proteins and 278 interactions was activated.

O4/P4. Glucocorticoid and B Lymphocytes Function: Role Of Glucocorticoid-Induced Leucine Zipper (GILZ)

Sara Flamini [1], Stefano Bruscoli [1], Francesco Adamo [1], Andrea Gagliardi [1], Oxana Bereshchenko [1] and Carlo Riccardi[1]

[1]Department of Medicine, Section of Pharmacology, University of Perugia, Perugia, Italy.

Glucocorticoid-induced leucine zipper (GILZ) is rapidly and invariably induced by Glucocorticoids (GC), which are the most commonly used drugs for treatment of autoimmune and inflammatory diseases. It has been previously shown that GILZ mediates many GC actions including anti-inflammatory effects. Recently, we have demonstrated that GILZ is as an important regulator of B cell survival and homeostasis. B cell activity has been linked to modulation of the inflammatory responses by antibody-dependent and independent mechanisms. Antibody-independent functions of B cells include production of the pro- and anti-inflammatory cytokines.

Here we show that lack of GILZ in B cells leads to an increased production of INFγ production not only in gilz cKO B cells, but also in co-cultured WT CD4+ T cells, thus suggesting that GILZ deficiency in B cells drives WT T cells toward a Th1-like phenotype. The transcriptional activation of INFγ promoter is due, at least in part, to an enhanced transcriptional activity of AP1 in GILZ cKO B cells. Moreover, we found that GILZ deficiency drives to an enhanced susceptibility to experimental colitis in mice and signs of the disease are more severe in gilz cKO B cells compared to WT. Interestingly, we have observed an increased production of INFγ in infiltrating B and T cells in lamina propria of cKO mice, indicating that GILZ contributes to the regulation of B and T cell functions in inflammatory processes during colitis.

1. Lack of Glucocorticoid-induced leucine zipper (GILZ) deregulates B cell survival and results in B cell lymphocytosis in mice. Stefano Bruscoli, Michele Biagioli, Daniele Sorcini, Tiziana Frammartino, Monica Cimino, Paolo Sportoletti, Emanuela Mazzon, Oxana Bereshchenko and Carlo Riccardi. Blood-2015-03-631580 Prepublished online August 14, 2015;

2. Glucocorticoid-induced leucine zipper (GILZ): a new important mediator of glucocorticoid action. Ayroldi E, Riccardi C. FASEB J. 2009 Nov;23(11):3649-58. doi: 10.1096/fj.09-134684. Epub 2009 Jun 30;

3. Identification of B-cell subsets: an exposition of 11-color (Hi-D) FACS methods. Tung JW1, Parks DR, Moore WA, Herzenberg LA, Herzenberg LA.Methods Mol Biol. 2004;271:37-58.

O5/P5. Improving the ability of hematopoietic stem and progenitor cells to generate microglia-like progeny upon transplantation

A. Montepeloso[1], F.J. Molina-Estevez[1], C. Baricordi[1], L. Biasco[1,4], M. Peviani[1], A. Biffi[1,2,3]

[1] Gene Therapy Program, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA, USA

[2] Gene Therapy Program, Department of Medicine, Boston Children's Hospital, Boston, MA, USA

[3] San Raffaele Telethon Institute for Gene Therapy, Division of Regenerative Medicine, Stem Cell and Gene Therapy, San Raffaele Scientific Institute, Milano, Italy

[4] UCL Great Ormond Street Institute of Child Health, London, UK

Recent findings indicate that hematopoietic stem and progenitor cells (HSPCs) can contribute to the turnover of resident brain myeloid cell populations, upon administration of a proper conditioning regimen. In the context of metabolic and neurological diseases, microglia-like cells replaced by the progeny of HSPCs can act as vehicles for therapeutics to the brain of affected patients as well as critical modulators of the inflammatory environment. However, the impact of this approach is affected by i) the lack of information concerning the nature and turnover of a functionally defined brain microglia precursor population (μP) and ii) the slow pace of reconstitution by HSPCs progeny, as compared with the rapid progression of the neurological disease.

With the goal of designing advanced HSPCs transplantation protocols, we provide a functional characterization of a bona fide μP, endowed with clonogenic potential, susceptible to Busulfan-based conditioning regimen and capable of engrafting upon transplant in hematopoietic tissues and brain of myeloablated recipients. This will be instrumental for targeting these cells specifically.

O6/P6. Effect of probiotics on Gardnerella vaginalis aggregation and adhesion to epithelial cells

Sabbatini S. [a], Monari C. [a] and Anna Vecchiarelli [a]

[a]Department of Medicine, Microbiology Section, University of Perugia, Perugia, Italy

Bacterial vaginosis (BV) is a disease that contributes over 60% to all vulvovaginal infections. It's associated with various disorders of the reproductive apparatus including infertility, many inflammatory diseases and, if present at early stages of pregnancy, preterm birth or spontaneous abortion. Recently, it has been shown that BV increases susceptibility to infections such as HIV, HPV, Chlamydia and Neisseria. BV is characterized by dysbiosis of the healthy vaginal flora resulting in an overgrowth of bacteria like Gardnerella vaginalis.

Because of the high rates of recurrences of BV, patients undergo to repeated antibiotic therapy and alternative approaches to prevent or to eradicate the infection are necessary. In this study the effect of three probiotic strains (Saccharomyces cerevisiae, Lactobacillus rhamnosus and Lactobacillus plantarum), on G. vaginalis aggregation and adhesion capacity on vaginal epithelial cells in an "in vitro" experimental model was evaluated.

The results obtained showed that some probiotics used were able to promote the aggregation of G. vaginalis. Furthermore, an inhibition of G. vaginalis adhesion to epithelial cells and a displacement of G. vaginalis when already adhered to epithelial cells was observed. In addition, preliminary results from in vivo experiments showed a therapeutic effect of probiotics during G. vaginalis infection.

O7/P7. The Effects of Drug Induced Phospholipidosis on Extracellular Vesicles Release

Lorena Urbanelli [1], Sandra Buratta [1], Krizia Sagini [1], and Carla Emiliani [1,2]
[1] Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy
[2] CEMIN-Center of Excellence for Innovative Nanostructured Material, Perugia, Italy

Drug-induced phospholipidosis (PLD), a transient intracellular accumulation of phospholipids into multilamellar inclusion bodies within late endosomal/lysosomal compartments, represents a major side-effect for many drugs. Amiodarone (AM) is a cationic amphiphilic drug (CAD) widely used as anti-arrhythmic agent and a well-known PLD inducer; most CADs induce PLD presumably by interfering with the late endosomal compartments. Membrane invagination of late endosome is known to originate Multivesicular Bodies (MVBs) containing up to hundreds Intraluminal Vesicles (ILVs), which can be released outside cells upon exocytosis ("exosomes"). Exosomes have been implicated in cell-to-cell communication in physiological and pathological condition. PLD could eventually influence the release of exosomes and, thus, modulate the transmission of vesicle-mediated signals to target cells. To unravel the connection between PLD and exosome release, we developed cell models that stably express the fluorescent fusion membrane proteins EGFP-CD63 and mCherry-CD63, resulting in the production of fluorescent vesicles. We, then, investigated AM-induced exosome release and showed that fluorescence could be directly assessed in cell culture medium. Isolation of exosomes by serial ultracentrifugation clearly indicated that AM treatment interferes with exosome release, suggesting an impairment of MVBs secretion. Instead of undergoing exocytosis, MVBs could collapse and contribute to the generation of PLD-associated multilamellar inclusion bodies.

O8/P8. Stem cell-Biomaterial interaction steers stem cells toward a selected specification lineage

Argentati,C.[a]; Morena, F.[a];Bazzucchi M.[a]; Montanucci, P.[b]; Armentano, I.[c]; Emiliani,C.[a,d]; Martino, S.[a]
[a] Department of Chemistry, Biology and Biotechnologies, University of Perugia; [b] Department of Medicine, University of Perugia; [c] Civil and Environmental Engineering Department, UdR INSTM, University of Perugia, Terni; [d] CEMIN-Center of Excellence for Innovative Nanostructured Material, Perugia, Italy

The tissue engineering strategies are based on the combination of a selected stem cell type, which has the ability to differentiate toward committed cell lineages, and a biomaterial, that, due to own characteristics, could serve as active scaffold to generate a bio-hybrid tissue/organ [1]. Within this issue, we are studying the molecular mechanisms underlying the stem cell-biomaterial interface. In this work we present data demonstrating how the active interaction between human umbilical cord matrix stem cells and poly (l-lactide) (PLLA) and PLLA / Multi Walled Carbon Nanotubes (MWCNT) films, ensures the formation of three-dimensional spheroidal structures and affects the fate transition of stem cells. The spheroids respond directly to a tunable surface and the bulk properties (electric, dielectric and thermal) of PLLA and nanocomposite PLLA/MWCNTs films by triggering a mechanotransduction axis. This phenomenon begins by binding the Focal Adhesion proteins of the cells to the surfaces of both films, and by the Adherens Junctions between the cells. These complexes transmit the forces to cytoskeleton proteins (to F-Actin stress fibres that link Filamin-A and Myosin-IIA proteins), generating a biological scaffold, with increasing stiffening conformation from PLLA to PLLA nanocomposite film, that modulate the nucloeskeleton proteins to boost chromatin reprogramming processes. Here, the opposite expression of NANOG and GATA6 transcription factors, together with other lineage specification related proteins, steer spheroids toward an Epiblast-like lineage commitment on PLLA films, and to a Primitive Endoderm [2].

1. Morena, F., Argentati, C., Calzoni, E., Cordellini, M.et al.Ex-Vivo Tissues Engineering Modeling for Reconstructive Surgery Using Human Adult Adipose Stem Cells and Polymeric Nanostructured Matrix, Nanomaterials, 2016, 6,4.

2. Morena, F., Armentano, I., Montanucci, P., Argentati, C. et al.Design of a nanocomposite substrate inducing adult stem cell assembly and progression toward an Epiblast-like or Primitive Endoderm-like phenotype via mechanotransduction, Biomaterials. 2017, 144, 211-229.

O9/P9. Proteases immobilization on innovative material for the degradation of agribusiness biomasses in White Biotechnology Applications

Eleonora Calzoni [a], Alessio Cesaretti [a], Alessandro di Michele [b], Silvia Tacchi [c], Silvia Caponi [c], Ilaria Armentano [d], Daniele Fioretto [b] and Carla Emiliani [a,e]

[a] Department of Chemistry, Biology and Biotechnology and CEMIN, University of Perugia; [b] Department of Physics and Geology, University of Perugia; [c] Istituto Officina dei Materiali del CNR (CNR-IOM) - Unità di Perugia, c/o Department of Physics and Geology, University of Perugia; [d] Department of Ecological and Biological Sciences, Tuscia University, Italy; [e] CEMIN-Center of Excellence for Innovative Nanostructured Material, Perugia, Italy

Proteases represent a family of enzymes able to catalyse hydrolysis of the peptide bonds between the amine and carboxylic acid group of proteins. It is the largest group of enzymes used in bio-industry by virtue of their high catalytic activity in a broad range of temperature and pHs, enabling industrial processes to shift to a more sustainable approach. In this work we have immobilized proteases on an innovative bio-material due to the growing interest in the use of immobilized enzymes, more stable than soluble enzymes, which can be used in continuous production processes. In particular, immobilized proteases do not undergo autolysis or denaturing processes[1]. We used proteases from Aspergillus oryzae that have been covalently immobilized on Poly-L-Lactic Acid by a method that uses an amine and glutaraldehyde to activate the support and make possible the binding of enzymatic molecules. We have carry out a biochemical characterization of the immobilized proteases, by evaluating the stability to temperature and pH variations, and physical characterization by Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and Raman Spectroscopy. Moreover, we evaluated the stability of the enzymatic activity over time and the ability of hydrolysing biomasses from waste agribusiness products.

[1]Zaborsky OR. Immobilized enzymes. Cleveland, Ott:CRC Press,1974

O10/P10. Candida biofilm: a multidisciplinary approach to gain insight on its structure and growth

Debora Casagrande Pierantoni [1], Martina Alunni Cardinali [2], Daniele Fioretto [2], Gianluigi Cardinali [1]

[1]Dipartimento di Scienze Farmaceutiche, Università di Perugia

[2]Dipartimento di Fisica e Geologia, Università di Perugia

The ability of many yeast species to form biofilm is a threat to the human health and a problem in the food industry where spoiler species can resist disinfection efforts by forming this structure. Biofilms are notorious for forming on different types of solid surfaces including implanted medical devices, catheters, pacemakers, heart valves, but also plastic materials and stainless steel, i.e. on most of the materials used to produce industrial machines and working surfaces. Candida albicans, for example, is a natural component of the human microbiome [1], but it is also the major fungal pathogen of humans for the persistence of the organisms on the devices on the biofilm form and makes them highly resistant to drugs and stresses. We used different approaches to analyse the biofilm forming ability and the biofilm structure of Candida albicans and non-Candida albicans Candida species (NCAC) strains. Thanks to the new joint Brillouin-Raman microspectroscopy (BMRS) technique we had an insight of the biofilm structure. Furthermore, a quantitative analysis has been carried out considering different parameters such as temperature, adhesion time and optical density, to elucidate the modality of biofilm growth.

1. S. Silva et al., Trends Microbiol 2011, 19, 241-247.

O11/P11. Involvement of the Aryl hydrocarbon receptor (AhR) in thyroid cell transformation

Menicali E. [1], Nucci N. [1], Morelli S. [1], Colella R. [2], Moretti S. [1], Puxeddu E. [1]
[1] *Dep. of Medicine, University of Perugia*
[2] *Dep. of Experimental Medicine, University of Perugia*

The Aryl hydrocarbon receptor (AhR) is overexpressed by many tumors and involved in the tumorigenic process. Kynurenine produced IDO1 in the tumor microenvironment, contributes to immune tolerance, motility and growth of cancer cells by binding AhR expressed by T lymphocytes and transformed cells. Very recently, we demonstrated that IDO is overexpressed by thyroid carcinomas.
Aim of our work was to evaluate expression and function of AhR in thyroid carcinomas.
AhR expression and function was evaluated in thyroid carcinoma samples and in human thyroid carcinoma-derived cell lines.
AhR resulted overexpressed in all thyroid carcinoma histologies compared to normal thyroid, either as mRNA or protein. A significant association between AhR and its transcriptional target CYP1B1 could be detected, indicating AhR functional activation in the tumor samples.
To study AhR function in thyroid cancer we selected FTC133 cell line that showed the coexistence of IDO and AhR overexpression and a spontaneous Kynurenine overproduction and we treated them with two agonists of the receptor, kynurenine and ITE. Both treatments resulted in a general activation of the receptor. At variance, kynurenire upregualted stemness markers, EMT markers, IDO and AHR expression and cellular motility while ITE downregulated them.

P12. Candida spp. identification by Raman microspectroscopy: the effect of rapid heating on the identification process.

Martina Alunni Cardinali [a]; Debora Casagrande Pierantoni [b]
[a] *Dipartimento di Fisica e Geologia, Università di Perugia, Via Pascoli, I-06123 Perugia, Italy*
[b] *Department of Pharmaceutical Sciences-Microbiology, University of Perugia, Borgo 20 Giugno 74, 06121 Perugia, Italy*

The rapid identification of pathogenic yeasts, as Candida, is foundamental in theraphy both to reduce the mortality rate due to nosocomial infections, both to fight the increasing problem of antifungal resistance. In recent years, Raman microspectroscopy has been used successfully for microbial identification, since it allows the direct analysis of early microcolonies grown on a solid medium culture. However this approach required both the subtraction of the medium contribution as additional step in the data elaboration process both the use of an high cofocal set-up which is, unfortunally, much more sensitive to sample heterogeneity. In this work we propose another approach to sample preparation that permits us to solve both problems. Four different Candida species were grown at 37°C for 24 hours, then they were washed twice from medium, centrifuged, pelleted and finally dried following two different procedures: a rapid heating at 42°C for 20 minutes and an air-drying at room temperature for about 5 hours. Results suggest not only that the different pellets can be distinguished by species but also that air-drying procedure provides better results since a rapid heating of the samples causes an increasing on fluorescence background, that strongly affect the spectral quality and the identification process.

P13. Effects of Hydrophobic Silver Nanoparticles on Gramicidin a Peptide Conformation

Marta Gambucci [a], Paola Sassi [a], Loredana Latterini [a]
[a] *Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia - Via Elce di Sotto, 8, 06123 Perugia, Italy*

A deeper knowledge of the effects of nanomaterials on protein conformation and folding is fundamental for the development of new therapeutic and diagnostic devices.Gramicidin A (GramA) is a

pentadecapeptide which can form ion channels inside phospholipid membranes, thus is an excellent model for membrane proteins and ion channels [1].In this work, the effect of silver nanoparticles (AgNPs) on GramA conformation into POPC liposomes is presented. Dodecanethiol-stabilized spherical AgNPs (D-AgNPs) [2] are prepared to have dimensions (5 nm) and an hydrophobic nature compatible with the POPC lipid bilayer.Tryptophan fluorescence and Raman signals were used to probe the position of the peptide inside the bilayer due to their sensitivity to the local environment [3,4]. Amide I and II vibrational bands in ATR-FTIR spectrum were employed to study GramA conformation [5]. Our results suggest that D-AgNPs may affect the peptide position in the bilayer and the formation of the ion channel.

1. D.A. Kelkar, A. Chattopadhyay, BBA-Biomembranes, 2007, 1768(9), 2011-2025.
2. S.Y. Kang, K. Kim, Langmuir, 1998, 14(1), 226-230.
3. J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 2006, Springer, New York.
4. P. Sassi et al. J. Raman Spectrosc., 2012, 43(2), 273-279.
5. A. Barth, BBA-Bioenergetics, 2007, 1767(9), 1073-1101.

P14. Study of solvation in poly(N-isopropylacrylamide) thermoresponsive microgel particles

Silvia Corezzi, Benedetta Petra Rosi, Lucia Comez

Many biologically relevant phenomena result from a competition between hydrophobicity and hydrogen bonding [1,2]. In mixed aqueous solutions cosolvents influence both types of interaction and promote biologically relevant actions. To minimize the complexity, the choice of a model amphiphile to mimic biological processes may be of help in the study of cosolvent-induced effects. One promising system consists of poly(N-isopropylacrylamide) (PNIPAM) microgel particles, nanometre-size polymer networks which respond to changes of temperature by undergoing a transition from a swollen to a collapsed state at a critical temperature (LCST) that can be regarded as an analogue of the cold denaturation in proteins [3]. UV-Raman scattering measurements have been used to get microscopic dynamical information to be correlated to macroscopic structural information obtained by PCS. As a preliminary result we have been able to detect vibrational signatures of the rearrangements occurring in the microgel structure during the volume phase transition, in particular the frequency shift of the peaks in the C-H bending and the amide region that are extremely interesting as they correlate with the size change of the particles.

[1] T. Wyttenbach, M. T. Bowers, Chemical Physics Letters 480, 1 (2009)
[2] I. M. Klotz, Protein Science 2, 1992-1999 (1993)
[3] Microgel suspensions: Fundamental and Applications, edited by A. Fernandez-Nieves et al., Wiley-VCH (2011)

P15. Novel combined gene/cell therapy strategies to provide full rescue of the Sandhoff pathological phenotype

Davide Sala [1,2], Francesca Ornaghi [1], Fabiana Tedeschi [1], Francesco Morena [2], Valeria Alberizzi [3], Alessandra Bolino [3], Sabata Martino [2] and Angela Gritti [1]
[1] IRCCS Ospedale San Raffaele, SR-TIGET, Milano, Italy
[2] University of Perugia, Perugia, Italy
[3] IRCCS Ospedale San Raffaele, Divisione di Neuroscienze, Milano, Italy

Sandhoff disease is a rare neurodegenerative lysosomal storage disorder caused by genetic defects in the b-N acetylhexosaminidase (Hex) activity, which leads to lysosomal accumulation of glycosphingolipids, glycoproteins and glycosaminoglycans, with devastating effects on the central nervous system (CNS) (Sandhoff et al., 1968). No treatments are currently available (Regier et al., 2016). Results from pre-clinical studies and clinical evidence stressed the importance of early intervention, high

levels of enzymatic reconstitution in the CNS and global targeting of affected tissues as fundamental requirements to obtain substantial therapeutic benefit. Here we will assess whether a clinically relevant combinatorial strategy based on neural stem cell transplantation (NSCT) or intracerebral gene therapy (IC GT) coupled to bone marrow transplantation (BMT) can supply timely and therapeutically relevant levels of functional Hex enzyme in the CNS and periphery of Sandhoff mouse model, thus preventing/delaying disease onset and progression, prolonging lifespan and correcting pathological hallmarks. Animals treated with single or combined approaches will be analysed to evaluate disease-associated hallmarks, their progression and extent of correction. Results of this study will assess the relative contribution of treatments to the therapeutic outcome in CNS and periphery of affected mice, with important implications for clinical translation of these innovative gene/cell therapy strategies.

P16. The leading role of the epithelial permeability in gut homeostasis

Marzia Sichetti [a], Donatella Pietrella [a], Giovanna Traina [a]
[a] Department of Pharmaceutical Sciences, University of Perugia, ITALY

Transwell model is widely exploited to recreate in vitro a functioning epithelial barrier. We studied the behaviour of different kind of epithelial cell lines from gastro-intestinal tract stimulated with ethanol or lipopolysaccharide (LPS) known to induce intestinal barrier disruption. The effects of this stimuli were evaluated measuring the transepithelial electrical resistance (TEER) using a millicell-ERS volt-ohmmeter (Millipore). Compared to non-treated controls, TEER of the cell monolayers treated with ethanol or LPS induce a significant decrease after 6h with a maximum of efficacy after 12h. Subsequently we used this transwell model to investigate the effects of a probiotic formulation (Serobioma) to modulate the inflammatory response of ex vivo monocyte-derived macrophages through the epithelial barrier. Human intestinal epithelial cell line HT-29 was cultured for 5 days on tranwell membranes to achieve fully monolayers and stimulated with LPS. After 24h of treatment with LPS, we notice an increase in the production of the anti-inflammatory cytokine (IL-10) and a down-regulation of the pro-inflammatory cytokine (IL-1β). Serobioma was able to modulate the inflammatory response of macrophages across the epithelial barrier. These data shed new lights on the hopefully use of probiotics as a coadjutant in the anti-inflammatory treatment of the gut epithelial barrier.

P17. Parameter estimation and identifiability analysis in Cancer Systems Biology: robust calibration of high dimension nonlinear dynamical models through omics data.

Lorenzo Tomassoni [1], Chiara Antonini [1], Paolo Valigi [1] and Fortunato Bianconi [2].
[1] Department of Engineering,University of Perugia, G. Duranti 95, 06132 Perugia; [2] Independent Researcher, Belvedere 44, 06036 Montefalco, Perugia, Italy e-mail: fortunato.bianconi@gmail.com (see http://www.fortunatobianconi.eu/).

In computational mathematical modelling, most model parameters are unknown because they can not be directly measured. Therefore, key issues in System Identification of nonlinear systems are model calibration and identifiability analysis. Existing methodologies for parameter estimation are divided in two classes: frequentist and Bayesian methods. The first ones optimize a cost function while the second ones estimate the posterior distribution of parameters. These methodologies suffer from an increasing computational cost with high dimensional models. Here, we present an innovative Bayesian method, Conditional Robust Calibration (CRC), for model calibration and identifiability analysis. The algorithm is an iterative procedure based on uniform and joint perturbation of the parameter space. At each step, CRC returns the probability density functions of parameters that progressively shrink toward specific points in the parameter space. These distributions are estimated on parameter samples that guarantee a certain level of agreement between observables and in silico measures. We apply CRC to a nonlinear

high-dimensional Ordinary Differential Equations model representing the pathway of p38MAPK in multiple myeloma. The dataset consists of proteomic cancerous data. We test CRC performances in comparison with profile-likelihood and a Bayesian algorithm. We obtain a more precise and robust solution with a reduced computational cost.

Dottorato in Biotecnologie
Università degli Studi di Perugia

Winter School on Biotechnology

26 January 2018 ore 17:30

Open day

Biotecnologie e Società

Sala della Galleria Nazionale dell'Umbria – C.so Vannucci

La Rivoluzione dei Big Data per lo scienziato e per tutti noi

Intervengono

Prof. Duccio Cavalieri
Università di Firenze

Prof.sa Flavia Marcacci
Università Lateranense

Mons. Tomasz Trafny PhD
Segretario Esecutivo Fondazione STOQ
Pontificio Consiglio della Cultura

Moderatore
Prof. Gianluigi Cardinali
Dottorato di Ricerca in Biotecnologie
Università degli Studi di Perugia

Regione Umbria
investiamo nel tuo futuro
www.regione.umbria.it/lavoro-e-formazione
ITS Umbria Academy
Iniziativa cofinanziata tramite il P.O.R. Programma Operativo Regionale F.S.E Fondo Sociale Europeo Umbria 2014-2020 e il Ministero dell'Istruzione, Università e Ricerca

Con il contributo di: