RSBMB 2015

25 Years of Promoting Molecular Life Sciences in Romania

International Conference of the Romanian Society of Biochemistry and Molecular Biology

Bucharest, 17-18 September 2015

BOOK OF ABSTRACTS

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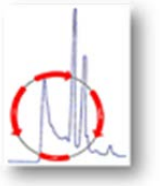

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Editors:
Stefana Petrescu, PhD
Elena Maria Antoneta Ganea, PhD

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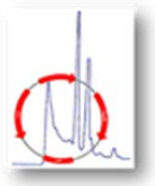

THE TOPICS OF THE CONFERENCE

- Opening Speech Conference
- Plenary Lectures
- Invited Speakers and Oral Presentations
- Workshop *„Molecular virology and clinical challenges in hepatitis treatment''* :
  - – Workshop Invited Speakers and Oral Presentations
  - – Workshop Posters
- Posters:
  - – *Protein Science*
  - – *Biochemistry and Molecular Medicine*
  - – *Nanostructures and biomedical applications*

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OPENING SPEECH CONFERENCE

- *Ştefana M. Petrescu*, President, RSMBM
- *Simona Vicas* - President Subsidiary RSBBM Oradea

[OSC 1] ROMANIAN SOCIETY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY. 1990-2015

Ştefana M. Petrescu, Ph.D

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Biochemistry and molecular biology have evolved in the last decade to the broader vision of understanding the molecular basis of life in an interdisciplinary way whilst translating this knowledge into health and technological applications to the benefit of the society.

The Romanian Society of Biochemistry and Molecular Biology (RSBMB) aims to promote this vision in Romania and hosts all those interested to contribute to the progress of this field and the dissemination of knowledge. RSMBM provides therefore the framework for debate and dialogue related to the structure, results and future of the Romanian molecular life science research and education by meetings, seminars, courses and publications. RSBMB aims also to create the necessary bridges between experts, authorities and the public to confront sensible issues and concerns generated by the advance of this scientific field.

RSBMB was founded in 1990 and was affiliated to the Federation of European Biochemical Societies in the same year. There was a long European tradition in Romanian biochemistry, as the Biochemistry Commission hosted by the Romanian Academy was a FEBS member since 1964. This year we celebrate not only 25 years from the birth of the new Society, but also 25 years of continuing FEBS support and collaboration.

The Romanian Society of Biochemistry and Molecular Biology (RSBMB) has benefited from a special support from FEBS, especially in the first difficult years for Eastern European science after the great political changes that began in 1989-1990. For example RSBMB has received a couple of visits from the Working Group on Assistance to Central and Eastern Europe (WOGCEE) and took advantage from the FEBS Program of Scientific Apparatus Recycling (SARP) innitated by Professor Peter Campbell, UK. In reciprocity RSBMB representatives have actively been involved in the development of FEBS activities by participating in supporting the activity of working groups of FEBS. For example Stefan Szedlacsek and Gabriela Negroiu have been members of WOGCEE, while Stefana

Petrescu was elected as a member of the Working Group on Women in Science (WISE) in 2004 and FEBS Advanced Courses in 2015.

However, by far, the most important consequence of the tight relation between RSBMB and FEBS was in boosting the scientiffic exchange and the access of Roumanian researchers to the FEBS education programs. As a result hundreds of young Romanian biochemists have been recommended by the RSBMB to obtain fellowships for participation at FEBS meetings and FEBS Advanced courses, while FEBS has selected RSBMB to organize three FEBS Advanced Courses that proved to be highly successful. WOGCEE visited Bucharest twice, once in 2000 and again in 2010. Both visits were aimed at evaluating the situation in molecular life sciences in Romania. Following the first visit, Romania was encouraged to apply to organize FEBS Advanced Courses and to hold educational workshops. All these activities contributed to the spreading of molecular biology among young scientists. The report from the second visit indicated that FEBS could offer experts for grant evaluation programs and recommended support activities to alleviate the brain drain of Romanian scientists to other countries.

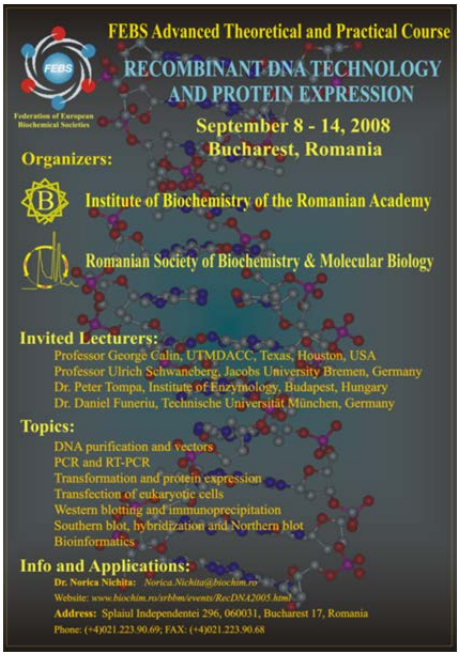
Biochemistry and molecular biology have thrived in these years in Romania and FEBS has played a significant part in building the special relationships which connected biochemists from different European countries through their science. Recombinant DNA technology has been widely adopted around the world as a standard tool. In Eastern Europe, however, there was still a lag in the extensive use of this powerful technique, and its advantages and applications in various research fields were not yet fully acknowledged. This was mainly due to the lack of infrastructure and flaws in the educational system, which still fails to provide a critical mass of qualified molecular biologists.

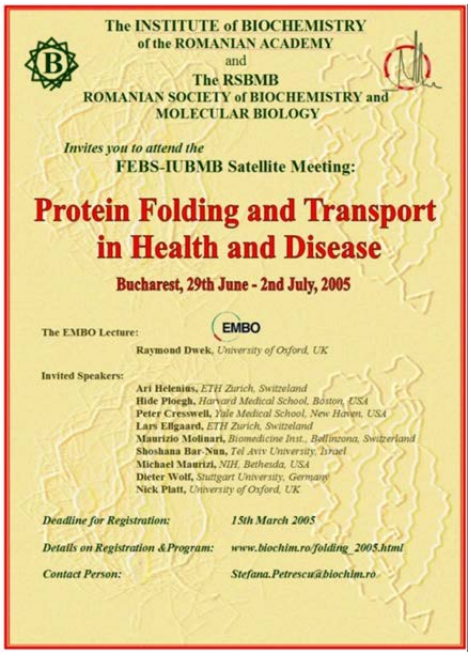
The FEBS Advanced Course '*DNA Recombinant Technology and Protein Expression*' organized by RSBMB was aimed at filling this gap. It comprised lectures and laboratory work, and was intended to benefit both beginners and those familiar with the basics of recombinant DNA technology. The course provided the participants with the essential principles and strategies of recombinant DNA technology, with a focus on the use of eukaryotic recombinant DNA, protein expression in eukaryotic cells, and specialized techniques and applications emerging from the opportunities created by recombinant DNA.

Organization of this course in Bucharest by the Romanian Society of Biochemistry and Molecular Biology helped young biochemists from Romania and neighboring Eastern European countries to acquire basic skills.

Romania proved to be an ideal location for the organization of this course owing to its position in Eastern Europe, which facilitated transport of participants from all surrounding countries. Moreover, the Institute of Biochemistry in Bucharest, where the course was held, has acquired experience during the last decade from organizing four FEBS Advanced Courses on this topic (in September 2001, September 2003, June 2005 and September 2008) with excellent results and appreciation from the participants.

Back home, RSBMB was instrumental in providing a platform for dissemination of research results and international scentific dialogue. Since 1990, besides the 25 Annual International Meetings held in Romanian major cities such as Bucharest, Cluj, Constanta, Oradea, Timisoara, RSBMB has organised many more conferences and workshops with invited speakers from all over the world. Among these we mention here the two Educational Workshops organized under the auspices of FEBS in Bucharest and Cluj; the 2014 Workshop "*Structure-guided investigation of effector function, action and recognition*", the 2005 FEBS Satellite Meeting "*Protein Folding and Transport in Health and Disease*", the 2004 Conference "*Glycosylation and Disease*", the 2003 mini-symposium "*Techniques in Biochemistry and Molecular Biology*", the 2000 FEBS Workshop "*Biochemical research in Eastern Europe*" etc.






FEBS-IUBMB Satellite Meeting
Protein Folding and Transport in Health and Disease
Bucharest, 29 June - 3 July 2005


FEBS
Federation of European Biochemical Societies

[OSC 2] SUBSIDIARY RSBBM ORADEA

Simona Vicas

University of Oradea, Faculty of Environmental Protection, Oradea Romania

Subsidiary RSBBM Oradea was founded in 2009, currently with 62 members from the Faculty of Medicine and Pharmacy, Faculty of Environmental Protection, Faculty of Science, from University of Oradea.

The most important event that held in Oradea subsidiary, has been the organization of The Annual International Conference of the RSBMB 2014 and Workshop "Viral hepatitis - from clinical to cell culture" during 5th- 7th June 2014, in collaboration with Institute of Biochemistry of the Romanian Academy, Romanian Society of Biochemistry and Molecular Biology and University of Oradea.

The International Conference was a great success, with 30 oral presentations, 60 posters, bringing together researchers in the field of biochemistry and molecular biology from the country (Oradea, Bucharest, Cluj Napoca, Craiova) and abroad (Switzerland, Hungary, France, Poland). Key speakers who have shared their experience were: Dr. Henning Schramm, Hiscia Institute, Society for Cancer Research, Switzerland, dr. Jozsef Prokisch, Univ. Debrecen, Hungary, Carmen Socaciu, Veterinary Medicine Cluj-Napoca.

The workshop was held by Jean Dubuisson, and Yves Rouille, Pasteur Institute of Lille, France and Costin Ioan Popescu, Institute of Biochemistry, Romanian Academy, Bucharest, Romania.

A premiere in the conference was the video-conference, where successfully presented by Octavian Henegariu, MD, Associate Research Scientist, Department of Neurosurgery Yale University School of Medicine with the following topic "Molecular identification and characterization of tools for mutations in neurogenetic Diseases".

During the conference were established new directions for research, new collaborations which will contribute to deepening the field of biochemistry and molecular biology.

PLENARY LECTURES

Vivek Malhotra - THE PATHWAY OF COLLAGEN SECRETION

Jean Dubuisson - HEPATITIS C VIRUS ENTRY INTO HEPATOCYTES

[PL 1] **THE PATHWAY OF COLLAGEN SECRETION**

Vivek Malhotra

Cell and Developmental Biology Program, Center for Genomic Regulation, 08003 Barcelona, Spain; email: vivek.malhotra@crg.eu

Almost four decades ago, George Palade uncovered a route by which eukaryotic cells secrete proteins to the extracellular space after synthesizing them in the endoplasmic reticulum (ER). This path requires secretory cargo to transit through the Golgi complex. Palade and coworkers reported the involvement of vesicular transport at two distinct junctions along the secretory pathway: small 50-nm vesicles operate between the ER and the Golgi complex, whereas large 500-nm secretion vacuoles (or granules) operate in the Golgi complex-to-plasma membrane (plasmalemma) step. In the past three decades, the use of genetic and biochemical approaches has led to a detailed understanding of how secretory proteins are sorted, packed into specific transport carriers, and delivered to their destinations within the cell and/or released in the extracellular space. The importance of these findings was recognized with the award of the Nobel Prize in Physiology or Medicine in 2013 to Rothman, Schekman, and Südhof for revealing the mechanism of vesicle-mediated transport. However, a challenge of considerable importance that remains poorly understood is the mechanism by which cells secrete bulky cargo such as collagen. This issue is fundamentally important, as collagens constitute approximately 25% of our dry body weight and are essential for almost all cell-cell interactions.

COPII vesicles mediate export of secretory cargo from the endoplasmic reticulum (ER). However, a standard COPII vesicle with a diameter of 60--90 nm is too small to export collagens that are composed of rigid triple helices of up to 400 nm in length. How do cells pack and secrete such bulky molecules? An answer to this question is now emerging by our revelation of a mechanism to pack procollagen VII (PCVII, the ER form of collagen) into a domain that produces a mega transport carrier. Packing is mediated by TANGO1, a protein that binds PCVII in the ER lumen via its SH3-like domain and interacts with COPII proteins SEC23/24 in the cytoplasm. Our new findings suggest that TANGO1 recruits ERGIC (ER-Golgi Intermediate Compartment) vesicles that fuse with ER to add membranes to produce a PCVII-containing mega export carrier. The mechanism for the biogenesis of a transport carrier required to export PCVII from the ER is therefore fundamentally different from the biogenesis of a standard COPII vesicle. I will discuss our new findings on this topic.

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[PL 2] **HEPATITIS C VIRUS ENTRY INTO HEPATOCYTES**

Jean Dubuisson

Institut Pasteur de Lille, Center for Infection & Immunity of Lille (CIIL), Inserm U1019, CNRS UMR8204, Université de France, Lille, France

Hepatitis C virus (HCV) is a small enveloped virus with a positive stranded RNA genome belonging to the *Flaviviridae* family. The virion has the unique feature to form a complex with a lipoprotein, which is called lipoviroparticle. Lipoprotein components as well as the envelope proteins, E1 and E2, play a key role in virus entry into the hepatocyte. HCV entry is a complex multistep process involving sequential interactions with several cell surface proteins. The virus relies on glycosaminoglycans and possibly on low-density lipoprotein receptor to attach to cells. Furthermore, four specific entry factors are involved in the following steps that lead to virus internalization and fusion in early endosomes. These molecules are the scavenger receptor SRB1, the tetraspanin CD81 and two tight junction proteins, Claudin1 and Occludin. It has also recently been shown that epidermal growth factor receptor act as host cofactors for HCV entry by regulating Claudin1-CD81 co-receptor association. Although they are essential to HCV entry, the precise role of all these molecules is not completely understood. Some recent data on HCV entry and virus interaction with cellular entry factors will be presented.

INVITED SPEAKERS
AND
ORAL PRESENTATIONS

[IS-OP1] **PROTEIN QUALITY CONTROL IN THE ENDOPLASMIC RETICULUM**

Ştefana M. Petrescu

Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, 060031, Romania

The endoplasmic reticulum (ER) of eukaryotic cells is a "lace-like reticulum" with a membrane network accounting for more then half of total cell membranes. Even since its discovery it has been a fascination for cell biologists in terms of morphology and cellular function. For biochemists, the ER is equally attracting because it harbors protein synthesis and transport to the Golgi. Our lab has been deeply involved in understanding the multiple molecular mechanisms evolved to protect nascent polypeptides starting with translocation from ribosomes to ER lumenal folding and targeted export. The encounter with Oxford glycobiologists inspired us further in pursuing the N-glycosylation of proteins starting co-translationally in the ER lumen. We have designed a model system to understand the way the ER can discriminate between folded, incompletely folded and terminally misfolded polypeptides and decide their fate. Tyrosinase, a transmembrane glycoprotein that is synthesized at the ER and exported to the Golgi and melanosomes in melanoma cells was our model established in a tissue culture system. Tyrosinase enabled us to understand that even if they are coded by DNA blueprints, proteins require further dedicated assistance from chaperones and folding enzymes resident in the endoplasmic reticulum to fold into the native structure. Using various experimental designs, including a number of 28 tyrosinase mutants, 15 stable cell lines and numerous antibodies developed in the lab we found distinct folding pathways for membrane versus soluble proteins. Some nascent chains engage into non-productive folding pathways that irreversibly lead to misfolded proteins. To avoid protein aggregation and maintain the protein homeostasis, the malfolded polypeptides are detected by the protein quality control and driven towards degradation. We followed protein degradation by the antigen processing and presentation processes underwent by tyrosinase as a tumor antigen. This lecture will focus on the molecular mechanism of misfolded proteins recognition and dislocation into the cytosol found to be common to a number of secretory proteins.

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[IS-OP2] **MODEL OF TRANSLATIONAL RESEARCH IN ONCOGENOMICS**

Irinel Popescu

Department of Surgery & Liver Transplant of Fundeni Clinical Institute, Bucharest, Romania

[IS-OP 3] **POLYMERS FOR DRUG/GENE DELIVERY AND TISSUE ENGINEERING**

Bogdan C. Simionescu

"Petru Poni" Institute of Macromolecular Chemistry of Romanian Academy, Iasi, Romania &Department of Natural and Synthetic Polymers, "Gh. Asachi" Technical University of Iasi, Romania

Recent years have witnessed an increased interest in the rational design of complex polymeric structures through new synthetic approaches. One of the main targets of this interest consists in the development of tailored polymer materials, engineered to exert distinct biological functions, implying multifunctionality as well as appropriate architectural features.

Several types of macromolecular structures including micelles, polymersomes, nano- and microparticles (-capsules/-spheres), dendrimers, nanogels, hydrogels, interpenetrated polymer networks have been developed and tested as potential systems of interest for bio-related applications. To gain clinical importance the new materials must provide not only high physic-chemical and biological performances but also processing characteristics. These demands often imply combination of natural and synthetic polymers or composite materials (inorganic/organic, biocomposites). The targeted application site or cargo may require specific material category (biodegradable, bioresorbable), dimension scale (micro/nano size) and topographic characteristics.

In this context, controlled drug delivery and its application in tissue engineering for tissue growth support and stimulation attracted much attention over the last decade, while combination of gene therapy and tissue engineering within a single system resulting in a powerful synergism of treatment options for regenerative medicine (scaffold mediated gene therapy) seems to be the favored alternative for tissue healing. Recent results make the domain very attractive, but key issues are to be solved to develop technologies of clinical impact.

The presentation summarizes the history and challenges in the discussed domains, pointing on polymers as a possible solution to specific challenges, and outlines the current state of the art, focusing on the newest strategies to improve systems effectiveness and responsiveness (design keys, preparative approaches). Expected future directions are underlined.

Acknowledgement. This work was financially supported by the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project PN-II-ID-PCCE-2011-2-0028.

[IS-OP 4] **COMPUTATIONALLY GUIDED RESEARCH IN MOLECULAR LIFE SCIENCE AT IBAR**

Andrei J. Petrescu, Adina Milac, Marius Micluţa, Lucian Spiridon, Marius Surleac, O. Căldărăru, Cristian V. Munteanu

Department of Bioinformatics and Structural Biochemistry, Institute of Biochemistry of the Romanian Academy - DBSB-IBAR, Splaiul Independenţei 296, 060031, Bucharest 17, Romania

The steep increase in computing power due to the advent of parallel high performance computing and the continuous advances of algorithms in string and structural bioinformatics and in molecular modelling and simulation have led lately to a significant improvement of our representation of biological systems at molecular level.

By combining computational techniques with constraints derived from various experiments we can now model the structure, interactions and dynamics of complex biomolecular systems comprising tens of thousands to millions of atoms.

In turn these models allow us to infer on the response of such systems to changes and test in this way hypotheses on biological processes and functions that can be then validated in the lab - replacing by this the pure empirical approach with a more focused model guided experimental research.

Established in 1999, the Department of Bioinformatics and Structural Biochemistry is the first in Romania to systematically develop such strategies and use them in our research programs. From the work performed so far we present here some of the more exciting results obtained on the investigation of structure-function relation in various protein families such as ion channels, proteins involved in DNA processing such as RAG1&2 and Topoisomerases or gene products involved plant pathogen interactions, as well as on early ER protein glycosylation and the consequences of this process on glycoprotein folding and structure.

[IS-OP 5] **ADIPOSE DERIVED STEM CELLS FOR SOFT TISSUE ENGINEERING**

B. Gălăţeanu, Marieta Costache

Department of Biochemistry and Molecular Biology, University of Bucharest, 91-95 Splaiul Independentei, Bucharest 050095, Romania

Background: Tissue engineering (TE) is a multidisciplinary field of research, which involves the use of biomaterials, growth factors, and stem cells in order to repair or to regenerate tissues damaged by injury or disease. TE applications such as regenerative medicine are currently focused towards the use of implantable biohybrids consisting of biodegradable scaffolds combined with *in vitro* cultured stem cells. This modern cell based therapy approach involves the design of 3D cell-scaffold bioconstructs in order to achieve *in situ* functional *de novo* tissue. Due to their particular secretory profile and ease harvest, human adipose derived stem cells (hADSCs) have been extensively used for regenerative and wound healing purposes. After implantation these cells may remain viable at the wound site and secrete growth factors in a continuous and regulated manner, just as it occurs in the natural wound healing process.

Methods: hADSCs were isolated from subcutaneous adipose tissue, harvested from female patients undergoing elective liposuction surgery [1]. After *in vitro* propagation, these cells were seeded in direct contact with polymeric composites designed for soft tissue reconstruction. Cell morphology and viability, as well as their proliferative status were investigated by fluorescence microscopy and spectrophotometric assays.

Results and conclusions: Several biomaterials were validated after their biocompatibility screening and differentiation potential and further *in vivo* studies will be employed on their base. MTT and Live&Dead assays revealed that natural polymers such as fibroin or cellulose combined with graphene oxide and mineral clays respectively sustained cell viability and proliferation. LDH spectrophotometric assay sustained these findings by revealing their low cytotoxic effect on hADSCs. Furthermore, our adipogenic and chondrogenic markers screening results show that gelatin-alginat-polyacrilamide biocompatible scaffold sustained both adipogenesis and chondrogenesis in hADSCs [2].

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Acknowledgements. This work was supported by the strategic Grant POSDRU/159/1.5/S/133391.

[IS-OP6] **IS CALORIC RESTRICTION THE WAY TO GO TO IMPROVE HEALTH, SLOW DOWN AGING AND POSSIBLY PROLONG LIFE?**

Ştefan A. Hulea

Pheromone Science Corp. Toronto, ON, Canada

The aging process is characterized by a progressive decline in multiple organ systems that affects reproductive, metabolic, physical and cognitive function and ultimately survival. It has two components: the primary, which is the inevitable deterioration of cell and tissue structure and function that occurs independent of lifestyle, disease and environmental influences and the secondary aging, i.e. the decline in tissue structure and function caused by disease and environmental factors.

Cross-sectional and longitudinal studies indicated that the obesogenic environment that is prevalent in the developed world and reduced physical activity are conducive to the development of metabolic syndrome, which includes obesity, type 2 diabetes, high blood pressure, hyperlipidemia, low-grade systemic inflammation, atherosclerosis and possibly cancer. These conditions have been linked to an early aging and a decreased life span. Animal studies and limited human studies have shown that caloric restriction that maintains proper nutrition is the only intervention to date that consistently decreases the biological rate of aging and the age-associated diseases. Thus, the beneficial effects of caloric restriction demonstrated in rodents, primates and in a few cases, in humans include: lower body weight and adiposity, lower core body temperature and resting energy expenditure, reduced thyroid hormone T3 plasma levels, low risk factors for cardiovascular disease such as high blood pressure, altered plasma lipid profile, high glucose and insulin levels. In addition, caloric restriction is associated with increased insulin sensitivity, decreased inflammation markers and glycation products as well as measures of oxidative stress. All these have a positive effect on human health and markedly diminish the risk of developing age-associated diseases.

Even if extending the human maximum life span is still a long way off the fact that by reducing the risk of age-related diseases we benefit by an improved quality of life and this is a plus that cannot be overlooked.

[IS-OP7] METABOLOMIC BIOMARKERS IN CANCER: FROM DISCOVERY TO DIAGNOSIS AND THERAPEUTIC MONITORING

Carmen Socaciu[1,2], Romanciuc Florina [1,2], Mihai Socaciu[2,3]

[1] *Dept. Biochemistry, Univ. Agr.Sci. &Vet. Med.;* [2] *RTD Centre of Applied Biotechnology in Diagnosis and Molecular Therapy, BIODIATECH ;* [3] *Univ. of Medicine & Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Romania*

Metabolomics, is a high throughput, emerging global metabolite analysis which proved to play an emerging role in cancer diagnosis and prognosis as well for the identification of novel biomarkers and developing therapeutics (1-3). Metabolomics is filling important gaps in the knowledge of cancer cell phenotype, metabolism and its atypical features in some specific metabolic pathways, understanding the fluxomic networks.

The review will focus on interconnected normal *versus* cancer metabolic pathways, key-metabolites found to be relevant as biomarkers by untargeted metabolic fingerprint and targeted profile, analytical and biostatistical strategies, implications on clinical diagnosis and tumor prognosis and therapeutic monitoring.

The results of emerging technologies like ultra performance liquid chromatography (UPLC) or gas-chromatography (GC) coupled with mass spectrometry (MS), or Magnetic Resonance Spectrometry (MRS) and their utility in cancer metabolomics will be described. The cancer global metabolome, determined in tissues and biofluids (serum, plasma, urine, saliva) will be characterized and also the key-biomarkers for a specific recognition of cancer evolution, diagnosis and prognosis (4,5). Particular attention will be payed to recently discovered biofluid biomarkers related to cancer diseases. Case studies related to breast and prostate cancer will be discussed to describe the metabolomics' steps from discovery to diagnosis and therapeutic guidance and to demonstrate the general utility of metabolomics in systems biology.

The key role of bioinformatics to achieve appropriate and relevant result interpretation of metabolomic data (PCA or cluster or heat map analysis) will be underlined to provide approaches for modeling therapies.

Updated databases for cancer metabolome and biomarkers will be discussed. The connection between the cancer supervision and future, personalized medicine based on specific metabolic profiles, related to epi-genetic and environmental factors, including the nutritional and "lifestyle" influence.

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[IS-OP 8] STUDIES OF BRANCHED POLYETHYLENIMINE LINKED TO A HYDROPHOBIC CORE AS GENE DELIVERY SYSTEM

Cristina M. Uritu[1], Manuela Călin[2], Stelian S. Maier[3], Bogdan C. Simionescu[1,3], Mariana Pinteală[1]

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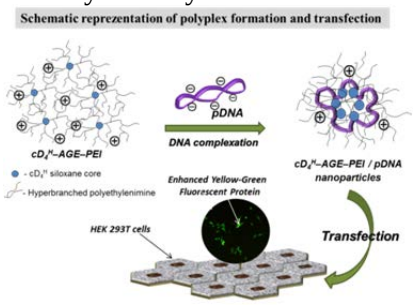
Over the passed decade, polyethylenimine (PEI), proved to be one of the most investigated polymers as non-viral vectors for gene delivery due to its very effectiveness in DNA/RNA packaging and release. It is known that PEIs of 5÷25 kDa are suitable for gene transfer; however they are leading to an increased cytotoxicity.[1] On the other hand PEI < 2 kDa is of great interest, being non-toxic, nevertheless with low transfection results. In order to enhance transfection efficiency and to keep a low cytotoxicity, the strategy is to couple together low molecular weight PEIs to a core yielding conjugates of 14÷30 kDa.[2] The core molecule should preferably possess several features: polyfunctionality, low cytotoxicity and a degree of hydrophobicity, a property which has been recently related with the increase of transfection efficacy.[3]

Herein we present the synthesis, characterization, and evaluation of transfection efficacy of a hybrid structure which comprises a siloxane ring (2,4,6,8-tetramethyl-cyclotetrasiloxane, cD4H) as hydrophobic core, and about 3.76 molecules of 2 kDa PEI as cationic branches. In silico molecular modeling and dynamic simulation show that the conjugate molecule (cD4H–AGE–PEI) tends to adopt an asymmetric structure, specific for amphipathic molecules, that favours a rapid interaction with nucleic acids. The polyplexes formation was confirmed by: zeta potential measurements, gel retardation assay and atomic force microscopy.



The conjugate and the polyplexes with the EYFP plasmid were proved to be biocompatible, and able to perform transfection in yields higher than 30 % on HEK 293T cells, compared with control reagent. The increased transfection efficacy originates in the ability of the conjugate to locally tightly encompass pEYFP molecules by electrostatic interaction mediated by the short PEI branches, and consequently to expose the siloxane hydrophobic moiety, which decreases the interaction energy with lipid layers.

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Acknowledgment. This work was financially supported by project PN-II-ID-PCCE-2011-2-0028.

[IS-OP 9] **BIOMEDICAL APPLICATIONS OF CHIP-BASED MASS SPECTROMETRY**

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In the last years, considerable efforts were invested in interfacing mass spectrometry (MS) to microfluidics-based systems as front end technology for electrospray ionization (ESI). Since its first introduction in biological mass spectrometry [1] chip-based ESI demonstrated a high potential to discover novel biopolymer species due to the efficient ionization, formation of multiply charged ions, minimization of the in-source fragmentation of labile groups attached to the biomolecular core and its elevated sensitivity [2]. In combination with high resolution mass spectrometers or instruments able to perform multistage fragmentation of chosen precursor ions, chip-electrospray confirmed its unique ability to offer structural elucidation at subpicomolar sensitivities of minor species in complex mixtures, which very often represent valuable biomarkers [3]. This aspect is of particular importance for the applicability of chip-MS approaches in biomedical and clinical investigation where only minute amounts of sample are usually available. In view of these major advantages of microfluidics in conjunction with modern MS instruments, our group developed, optimized and introduced in biomedical research chip-based ESI in conjunction with either ion trap or QTOF MS for glycoscreening and sequencing. The present paper will focus on the strategies, which allowed a successful application of chip technology for glycopeptide, glycolipid and glycosaminoglycan mapping and sequencing, for identification and structural analysis of the glycan species expressed different normal human matrices and those associated to severe pathologies as well as for studying protein-glycan functional interactions. Hence, chip-based ESI MS and multistage MS approaches were developed for glycoconjugate analysis in brain pathologies *i.e.* neurodegenerative diseases, primary and secondary brain tumors, extracellular matrix and body fluids for biomarker discovery, and detailed structure elucidation of disease-associated species. The method accomplishments in characterization of novel structures indicate that advanced chip-based MS has real perspectives to become a routine method for early diagnosis based on determination of molecular fingerprints.

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[IS-OP 10] **BIORESPONSIVE MATERIAL SURFACES FABRICATED BY INNOVATIVE LASER APPROACHES**

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Material processing and engineering are current strategies for discovering new solutions for potential biological use. Material choice and design stand for the initial approaches when the research meets a concrete application. Biomaterials are often expensive and difficult to configure within micro- and nano-spaces.

Laser technologies in 2D and 3D environments compensate the need of substitution at small dimensions. By processing at micro- and nano-scale, lasers emerged as versatile tools for fabricating innovative devices for tissue engineering, biomimetic delivery systems for local release or lab-on-chip applications.

Herein, laser based methods will be reviewed as challenging technologies for chemical methods for surface modification with organic and composite materials' assembling [1-4]. By adapting the irradiation conditions with thermo-physical properties of materials, new hybrid organic-inorganic nanoscale structures with improved biological properties were fabricated [5, 6]. The processed biomimetic surface-cell interfaces are evaluated as potential synergistic environments for tissue engineering and local release applications. Laser direct writing technologies will be considered as tools for fabricating optofluidic devices with micro-scale dimensions. Mixed complex inorganic-organic 3D material configurations will be discussed as potential scaffolds for monitoring cancer single cell migration, orientation or invasiveness.

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[IS-OP 11] NANOPARTITICLE BASED DRUG DELIVERY SYSTEM AND THE EFFECT OF PARTICLE DESIGN ON THEIR BIOCOMPATIBILITY INTRACELLULAR FATE

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The main objective in the area of Nanomedicine and Drug Delivery Systems (DDS) is to design a suitable pharmaceutical nanocarrier with controllable properties for drug delivery and site-specific targeting to achieve greater efficacy and minimizing the toxic effects compared to the conventional drugs. Lipid nanostuctures (LNS) offer several advantages over other delivery systems, including perfect biocompatibility, posibility to control the physicochemical properties in order to improve the specificity of interaction with biological milieu. During many years we have developed intensive research in liposome technology in order to design biocompatible lipidic nanocarriers, liposomes, suitable to encapsulate efficiently bioactive molecule. Different LNS systems were obtained and characterised in term of size, polidispersity index, lamellarity and zeta potential and for two of them we have obtained patents. Their biocompatibility, stability in the biological medium and ability to modify cell function were tested *in vitro* (different cell culture- fibroblasts, chondrocytes, monocytes, dendritic cells) and *in vivo* (DBA1 mice with induced rheumatoid arthritis) experiments.

On the other hand polymeric nanoparticles (PNPs) are known to facilitate intracellular uptake of drugs to improve their efficacy, with minimum bioreactivity. Based on our expertise with LNS we have studied biocompatibility and intracellular fate of PNPs in different cell culture (mouse melanona cell- B16-F10, human hepatoma cell- HepaRG and two epithelial cell lines, MDBK - from bovine kidney and Colo 205 - from human metastatic colon). Our data demonstrated that PNPs were stable in PBS at 4 ºC for 30 days and not cytotoxic to the studied cells at concentrations up to 2500 µg /mL. Like in the case of LNS, the surface charge of PNPs influenced the cellular uptake and their intracellular fate. This study offers new insight on PNPs uptake and localization within target cells supporting the use of PNPs as a novel nutraceuticals/drug delivery system in metabolic disorders or cancer therapy.

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[IS-OP 12] IDENTIFICATION OF MAJOR EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MUTANT GENOTYPES IN NON-SMALL CELL LUNG CANCER ROMANIAN PATIENTS

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Introduction. Approximately 15-20% of patients with NSCLC have tumor associated *EGFR* mutations. These mutations occur within EGFR exons 18–21, which encodes a portion of the EGFR kinase domain. These mutations increase the kinase activity of EGFR, leading to hyperactivation of downstream pro-survival signaling pathways. In the era of molecular and personalized therapeutics, the discovery of mutations in epidermal growth factor receptor (EGFR) in lung adenocarcinomas and the associated response to EGFR-targeting tyrosine kinase (TK) inhibitors have provided a successful avenue of attack in high-stage adenocarcinomas**.** Initial studies with the EGFR tyrosine kinase inhibitors (TKIs) gefitinib (Iressa) and erlotinib (Tarceva) demonstrated biologic and clinical activity in only a relatively limited subset of lung cancers. Further investigation demonstrated that the highest response rates to these TKIs were seen in patients with somatic mutations within the EGFR-TK domain, particularly exon 19 deletion, exon 21 L858R, and exon 18 G719X. By contrast, the exon 20 T790M mutation is associated with acquired resistance to TKI therapy. In a small scale analysis, we screened for such mutations in NSCLC Romanian patients.

Materials and Methods. We performed two independent genomic DNA extractions from each of the 124 analyzed paraffin embedded lung tissues collected from NSCLC patients. Using either Beckman Coulter GenomeLab CEQ 8800 Genetic Analysis Systems or Qiagen's PyroMark Q24 system we looked for variations within particular mutational hot-spots in EGFR exons 19 and 21. Sequence analyses were performed using manual annotation, specific sequencing system software and UCSC Genome Browser.

Results. We found that 18 percent of the patients carry mutations that are known to be associated with a good response to TKI based cancer therapy. More than 80 percent of them are located in exon 19, the most preeminent being delE746-A750. The other mutations are located in exon 21 and fall within the L858 somatic mutations category.

Conclusions. Our study revealed a relative small number of EGFR mutations, which may be incidental to a relatively small number of analyzed patients. The most frequent mutations that we identified are also reported to be world-wide most frequent, as stated in EGFR Mutation Database. We also found previously unspecified mutations within exon 19 residing in the corresponding mutational hot-spot.

[IS-OP 13] **RA-WNT-FGF SIGNALING INTERACTIONS DURING NEURAL TUBE CLOSURE IN MOUSE EMBRYOS**

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Retinoic acid (RA) is essential for axial elongation and morphogenesis of vertebrate embryos. RA deficient embryos exhibit axial truncation, with asymmetric somites and an open neural tube and die around E9.5 due to severe cardiac defects. However, the molecular mechanism underlying the neural tube closure defect is not known. Here we show that during neurulation stages, RA generated in the mesoderm controls the interactions between Wnt signaling and FGF signaling pathways at the level of neural ectoderm.

WORKSHOP

„MOLECULAR VIROLOGY AND CLINICAL CHALLENGES IN HEPATITIS TREATMENT''

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- **WORKSHOP INVITED SPEAKERS AND ORAL PRESENTATIONS**
- **WORKSHOP POSTERS**

WORKSHOP INVITED SPEAKERS AND ORAL PRESENTATIONS

[W.IS-OP 1] **MORPHOLOGY AND MOLECULAR COMPOSITION OF PURIFIED BVDB PARTICLES**

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The family Flaviviridae includes viruses that have very different virion structures and morphogenesis mechanisms. Most cellular and molecular studies have been so far performed with viruses of the Hepacivirus and Flavivirus genera. Here, we studied bovine viral diarrhea virus (BVDV), a member of the Pestivirus genus. We set up a method to purify BVDV virions and analyzed their morphology by electron microscopy and their protein and lipid composition by mass spectrometry. Cryo-electron microscopy showed near spherical viral particles displaying an electron-dense, apparently poorly structured capsid surrounded by a phospholipid bilayer with no spikes. Most particles had a diameter of 50 nm and about 2% were larger with a diameter of up to 65 nm, suggesting some size flexibility during BVDV morphogenesis. Morphological and biochemical data suggest a low envelope glycoprotein content of BVDV particles, E1 and E2 being apparently less expressed than Erns. Lipid content of BVDV envelope displayed a ~2.3 fold enrichment in cholesterol, sphingomyelin and hexosyl-ceramide, concomitant with a 2.1 to 3.5 reduction of all glycerophospholipid classes, as compared to lipid content of MDBK cells. This suggests that BVDV morphogenesis includes a mechanism of lipid sorting. Functional analyses confirmed the importance of cholesterol and sphingomyelin for BVDV entry. Surprisingly, despite a high cholesterol and sphingolipid content of BVDV envelope, E2 was not found in detergent-resistant membranes. Our results indicate that there are important differences between the molecular compositions of viral particles of Flaviviruses, Pestiviruses and Hepaciviruses, and suggest that they use different mechanisms for their morphogenesis.

[W.IS-OP 2] **NOVEL FUNCTIONS OF THE ENDOPLASMIC RETICULUM ASSOCIATED DEGRADATION IN THE HBV LIFE-CYCLE**

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Infection with Hepatitis B Virus (HBV) is a major health problem, affecting about 240 million people worldwide. Following virion attachment to hepatocytes, the envelope is removed and the naked nucleocapsids containing the partially double stranded DNA (pdsDNA) genome are trafficked to the nucleus. The pdsDNA is converted into covalently closed circular DNA (cccDNA), a stable replication form which serves as template for transcription of pregenomic and subgenomic mRNAs. So far, there is no treatment to prevent cccDNA formation or cure it once it is formed. The mechanism of cccDNA synthesis, regulation and amplification is not well characterized, despite its importance in the antiviral therapy. Previous data have indicated a potential role of the envelope proteins in regulating cccDNA formation. Here, we show that members of the endoplasmic reticulum (ER) degradation-enhancing α-mannosidase-like proteins (EDEMs) are significantly upregulated in HBV-replicating cells. These proteins have contrasting effects on the HBV envelope proteins, promoting degradation of small (S) and large (L) and rapid extracellular export of middle (M) proteins. We hypothesize that efficient disposal of the HBV envelope proteins by EDEMs, could play a role in cccDNA turnover, early in infection. To investigate this possibility we have constitutively modulated EDEM proteins expression in HepaRG cells, which are permissive for HBV infection *in vitro*. The newly established cell lines were characterized biochemically and functionally. Interestingly, HBV infection in EDEM3 overexpressing cells resulted in a significant increase of the amount of nucleocapsid DNA and enhanced secretion of virus particles. The level of HBV-specific transcripts was also higher in these cells compared to controls, which correlated with the increased number of cccDNA copies/cell. We propose that the EDEMs-induced removal of the envelope proteins is a contributing factor to stabilization of the cccDNA replication form, by favouring the nuclear recycling of HBV nucleocapsids at early stages of infection.

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[W.IS-OP 3] INVESTIGATION OF HEPATITIS C VIRUS – HOST INTERACTIONS USING INTERACTOMICS AND FUNCTIONAL GENOMICS TECHNIQUES IN INFECTIOUS VIRIONS PRODUCING CELLS

Ovidiu Vlaicu[1], Florin Pastrama[1], Cristian Munteanu[1], Leontina Banica[2], Simona Paraschiv[2], Laura Riva[3], Yves Rouille[3], Jean Dubuisson[3], Costin-Ioan Popescu[1]

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Hepatitis C Virus (HCV) infection has profound effects on the hepatocyte transcriptome, lipidome and proteome. The direct interaction between the viral proteins and the endogenous factors begins to reveal the molecular mechanisms of HCV – host interaction. While the entry and replication host factors were extensively investigated, there are few edogenous factors reported to be involved in HCV assemby. In order to identify host factors potentially involved in HCV assembly, we used the HCV cell culture system and affinity purification (AP) coupled to mass spectrometry analysis (MS). We focused our analysis on three viral proteins involved in HCV assembly: NS2, p7 and NS5A. Affinity purification suitable tags were fused to the viral proteins in the context of infectious recombinant viruses and AP-MS anaysis was performed using infectious virions producing cells. PI3KC2α was identified as an interactor of the NS2 complex. We showed that PI3KC2α is involved in genome replication, but not in entry or assembly and secretion steps. In parallel, the NS2, p7 and NS5A interactor lists were correlated with a whole genome functional screen to select host factors to be confirmed in secondary screen for their role in HCV assembly step. The analysis of the role of the selected targets in HCV life cycle is in progress. Our strategy of combining proteomic and functional genomics approach has the potential to identify new host factors involved in HCV life cycle which will expand our understanding of HCV – host interaction and may help in finding new biomarkers for liver injury progression.

[W.IS-OP 4] **A NOVEL HEPG2-CD81 POLARIZED CELL CULTURE MODEL TO STUDY THE ROLE OF CELL POLARIZATION IN HCV INFECTION**

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HCV infects primarily hepatocytes that are highly polarized cells with a complex organization. In recent years, the knowledge concerning HCV cell entry has improved, however the relevance of cell polarity in HCV life cycle has been addressed in distant models and remains unclear. A physiologically relevant hepatocyte model is required to investigate HCV life cycle in polarized cells. The plasma membrane of polarized cells is divided into specialized domains with different functions and lipid and protein compositions. Although polarized epithelial cells have a rather simple morphology with one basolateral domain and one apical domain, hepatocytes exhibit complex polarization structures. However, it has been reported that some selected polarized HepG2 cell clones can exhibit a honeycomb pattern of distribution of the tight junction proteins, typical of columnar polarized epithelia which can be used as a simple model to study the role of cell polarization in viral infection of hepatocytes (Snooks et al., *J Virol* 2008, 82, 8733). To obtain similar clones, we used HepG2 cells expressing CD81 available in the laboratory and we isolated clones by limiting dilution. To validate the polarization properties of the isolated clones, we analyzed the localization of apical, basolateral and tight junction markers. The functionality of the polarization was assessed in secretion assay of human serum albumin. We isolated and characterized two clones with good polarization capacity when grown on semi-permeable support. To test the polarity of HCV entry and release, our polarized clones were infected with HCVcc. Our data indicate that HCV binds equally both side of the cells, but productive infection occurs mainly at the basolateral domain. Furthermore, we also observed that the virus is secreted at the basolateral domain of the cells. This cell culture system provides an useful model to study the influence of cell polarization on the HCV life cycle.

[W.IS-OP 5] MOLECULAR EPIDEMIOLOGICASL STUDY TO ESTIMATE THE DATE OF HCV AND HIV INTRODUCTION IN ROMANIAN INTRAVENOUS DRUG USERS

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Background: HIV cases among Romanian intravenous drug users (IDUs) significantly rose in recent years. Ninety percent of these patients are HCV co-infected. The aim of the study was to estimate the date of introduction of HIV and HCV into this risk population using molecular phylogeny approaches.

Methods: 117 IDUs newly diagnosed with HIV and HCV co-infected were analysed. Thirty-three HCV mono-infected patients, non-IDUs, were also included in the study. The NS3 and NS5b regions of HCV and the pol gene of HIV (1300bp) were sequenced. Molecular clock analyses of HIV/HCV sequences were performed using a Bayesian approach as implemented in BEAST version 1.8.1.

Results: 68% of IDUs were infected with subtype F1 viruses and 26% with CRF14_BG and recombinants of CRF_14 and F1. Three HIV transmission networks (2 F1 subtype and one CRF14_BG) were identified. The estimated time to the most recent common ancestor (tMRCA) for CRF14_BG cluster was 2007 (95% Higher Posterior Density HPD: 2004-2010). HCV genotype distribution among IDUs was more diverse: genotype 1a is the most frequent (48 patients), followed by 1b (40), 3a (14), 4d (8) and 4a (7). Molecular clock analyses indicated similar tMRCA for genotypes 3a, 4a and 4d: 2010 (HPD: 2007-2012). Genotype 1a was introduced 10 years ago into this risk population (tMRCA: 2003, HPD: 1999-2007). Genotype 1b was estimated to be introduced 25 years ago into IDUs risk gropup and up to 40 years in non-IDU patients.

Conclusions: According to phylogenetic analyses, HIV and HCV transmission occurred independently in Romanian IDUs. Overall, HIV and HCV genotypes 3a, 4a and 4d were recently introduced in this risk population, whereas infections with genotypes 1a and 1 b are older.

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WORKSHOP POSTERS

[W.P 1] **LIVE IMAGING OF HEPATITIS C VIRUS NS5A AND CORE PROTEINS IN A *TRANS*-COMPLEMENTATION SYSTEM**

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Hepatitis C Virus (HCV) NS5A protein is a multifunctional protein involved in both assembly and replication steps in the virus life cycle. Moreover it represents an essential drug target in the actual direct acting antiviral (DAA) therapy aganist HCV. The precise role of NS5A protein in the viral life cycle is still elusive. Previous reports showed that NS5A exists in two distict subcellular populations with different mobilities: small puncta capable of long saltatory movments and larger, imobile dotted structures. The role of the two NS5A populations and their intracellular dynamics are currently unknown. In order to investigate the role of NS5A movements in HCV life cycle, we developed a *trans*-complementation system suitable for life imaging of NS5A and core proteins. The system consists of a subgenomic replicon lacking core which has an in frame fusion between a fluorescent protein (EGFP or mNeptune) and the NS5A protein. A tagged core suitable for „*in vivo*" labeling was able to *trans*-complement the subgenomic replicon comparable as efficacy with the tagged core *in cis*. Using this system, triple labeling was performed for core, lipid droplet and NS5A protein. We identified the two NS5A populations and we evaluated the influence of core expression for the NS5A mobility. As expected, the core expression relocalized NS5A protein in a perinuclear region in LD proximit. The small NS5A puncta in perinuclear region were less defined and mobile than those at the cell perifery. This *trans*-encapsidation system will be a valuable tool in studying the „*in vivo*" dynamics of viral proteins and endogenous factors from HCV life cycle.

[W. P 2] HIGH THROUGHPUT SCREENING ASSAY FOR IDENTIFICATION OF NEW CHEMICAL SCAFFOLDS WHICH INHIBIT HCV NS2 CYSTEIN PROTEASE ACTIVITY

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Hepatitis C Virus (HCV) represents a serious global health problem with 170 million people being infected worldwide. The standard of care has been recently upgraded with direct acting antivirals with or without interferon and ribavirin. Despite impressive sustained virologic responses across all genotypes and liver conditions, the drug resistance and genetic variability of the virus still represent issues to be addressed. The current therapy relies on molecules targeting the NS3 protease, the NS5B polymerasse and a multifunctional protein NS5A. Besides the NS3 protease, the other viral protease NS2 was validated as an attractive drug target being involved in assembly and indirecty in viral replication by NS2-NS3 cleavage. The identification of specific inhibitors of NS2 cysteine protease activity was hampered by an automatable assay. We have optimized a cell based assay for NS2 genotype 2a which was further miniaturized and automatized in 384 well plate format. The assay was validate with a Z'>0.5. Using „in silico" docking techniques, we selected 2000 potential cysteine protease inhibitors which were subsequently clustered based on structural and functional molecular properties. A subset of 320 diverse compounds has been selected and further submitted to NS2 protease activity determination. A primary screen was performed and different hits were identified. Moreover, an assay for specificity was developed and compound toxicity was evaluated in order to remove the false positives. The novel cell based assay for NS2 protease activity prove very useful to show that HCV NS2 protease is a druggable target.

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[W. P 3] **A NOVEL PEPTIDYL-ACYLOXYMETHYLKOTNE AS ACTIVITY BASED PROBE FOR CYSTEINE PROTEASES**

Anca G. Coman[1], Codruţa C. Paraschivescu[2], Andrei Juncu[3], Ovidiu Vlaicu[3], Niculina D. Hadade[2], Costin-Ioan Popescu[3], Mihaela Matache[1]

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Cysteine proteases (CP) are enzymes involved in numerous biological procesess like apoptosis and the immune responce. Moreover, RNA positive viruses beloging to various genra like flaviviridae or picornaviridae code in their genome for cysteine proteases which are essential for their replication. In order to understand the diverse biological roles of CPs, activity based probes (ABPs) may be used as chemical tools. ABPs are usually synthetic small molecules which react selectively and covalently in the active site of active enzymes. The synthesis of peptidyl-acyloxymethylketone - an efficient class of ABPs - is cumbersome, involving both solution and solid-phase organic synthesis, and it depends on the target aminoacids sequence. Thus, our aim was to optimize a general method for the synthesis of the peptidyl-AOMKs on solid support. In addition, we report the design and synthesis of a new acyloxymethylketone derivative able to be attached on a solid support which allows the subsequent growth of any required peptide sequence. The IC50 for cathepsin B inhibition was further determined for the new probe, providing similar results to well-known ABPs specific for cathepsin B. This method facilitates the synthesis of new peptidyl-AOMK probes to be used for the investigation of the biological roles of both viral and endogenous cysteine proteases.

[W. P 4] GENOTYPING TELAPREVIR RESISTANCE AND PHENOTYPING OF THE RESISTANCE MUTATIONS IN HEPATITIS C VIRUS CELL CULTURE SYSTEM

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Hepatitis C Virus (HCV) infects 170 million people worldwide determining liver disease ranging from steatosis to chirosis and hepatocellular carcinoma. Recently, the golden standard therapy relying on alpha interferon and ribavirin was profoundly improved with the Food and Drugs Administration (FDA) approaval of direct acting antivirals (DAA) alone or in combination with interferon and ribavirin. Although the efficacy of the new standard of care is quite high, drug resistance and viral genetic diversity are still issues which require further investigation. We started our study with genotype 1b patients who failed to achieve sustained virological responce (SVR) after treatment with triple therapy telaprevir, alpha interpheron and ribavirin. The telaprevir resistance was genotyped by population sequencing in the NS3 protease region. Interestingly, we found double lower level resistance mutations (T54S, R154S, A156S) associtated with elevated viral loads suggesting a minimal effect on the viral fitness. Further, we used the HCV cell culture system and a 5'UTRNS5A1a/2a chimeric virus to phenotype the resistance mutations identified in patients. The replication and secretion capacity were evaluated by anti-core ELISA and qRT-PCR. The infectivity was assesed by titration using the foci forming unit assay. Possible compensation mechanisms between drug resistance mutations are further discussed. The strategy presented herein may contribute to our understanding of drug resistance mechanisms in the emerging interferon free therapy era.

[W. P 5] COUMARIN LABELING OF MIDDLE PROTEIN OF HEPATITIS B VIRUS

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Hepatitis B virus (HBV) is an enveloped, DNA virus and a member of the *Hepadnaviridae* family, with a pronounced tropism for hepatocytes. The viral DNA is packed inside a nucleocapsid surrounded by a lipid membrane, which contains three envelope proteins: large (L), middle (M) and small (S) with distinct functions in assembly, secretion and infection.

The aim of our work was to fluorescently label the viral envelope to investigate the mechanism underlying early steps of HBV infection. As the M envelope protein was shown to be dispensable for HBV infectivity and assembly, we hypothesized that additions of tags would not affect these processes. Therefore, the M protein was considered as target for fluorescent labeling of the virus particle.

Previous studies in our group have shown that recombinant enhanced green fluorescent protein (EGFP)-labeled M protein (EGFP.M) was assembled into mature HBV envelope. The proper secretion of virions was achieved only in the presence of S protein. In this study we have constructed a mutant M protein (MN3) containing a 13 amino acids sequence, which is a specific tag for the PRobe Incorporation Mediated by Enzyme (PRIME) labeling using an engineered fluorophore ligase (LplA) from *E.coli* and the blue fluorophore, coumarin. We have purified from bacterial systems the recombinant LplA using FPLC with Ni- and glutathione –Sepharose columns.

Preliminary results demonstrated that the recombinant MN3 protein is efficiently expressed, folded and secreted in hepatoma cells. Transcomplementation experiments using MN3 and HBVΔM, an M-deficient HBV, showed that the MN3 expression does not affect either HBV replication or HBsAg secretion. Further studies regarding the infectivity features of the recombinant MN3_HBV virus are currently in progress.

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[W. P 6] ANTI-HEPATITIS B VIRUS ACTIVITY OF LACTOFERRIN-DERIVED PEPTIDES

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Lactoferrin (Lf) is an iron binding glycoprotein which features a variety of biological properties, including immunomodulatory and antiviral activity. It was shown that two glycosaminoglycans (GAGs) binding sites located in the N-terminal (N-t) region of Lf are involved in its antiviral activity, one of them being a cationic cluster (GRRRR).

Previously, we have demonstrated that four human Lf (HLf)-derived peptides (HLP), corresponding to the N-t domain of the native protein (1-47 amino acids sequence), inhibited hepatitis B virus (HBV) infection between 40 to 80% . The most potent inhibitor was HLP_{1-23}, a peptide containing the GRRRR cationic cluster which prevented HBV infection by neutralizing the viral particles. In this study, we aimed to improve the antiviral activity of this peptide using computer modelling followed by chemical synthesis. With the purpose of enhancing interaction with the cell surface GAGs, we designed a new mutant peptide with increased overall positive charge and aromaticity, supposedly displaying improved affinity through an additional GAGs binding site and increased stability through supplementary aromatic stacking interactions. The newly designed peptide HLP_{1-33}, was assayed for potential cytotoxicity in HepaRG cells. The results have shown that the peptide revealed good solubility in aqueous solution and no toxicity for concentrations up to 100 μM. This enables further studies of the antiviral properties of the newly designed peptide, in both HBV infectivity and replication cellular systems, which are currently under investigation.

Acknowledgements. This work was supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390 and by Romanian Academy Project 3 of the Institute of Biochemistry.

[W. P 7] **NOVEL HBV ANTIGENS FOR COST-EFFECTIVE PRODUCTION IN PLANTS**

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Hepatitis B Virus (HBV) infection remains a major global health problem. Despite the availability of a safe and efficient commercial vaccine, its use in developing countries is hampered because of the costs of mass vaccination programs. In addition, 10% of the vaccinated individuals fail to develop a protective immune response to current vaccines against HBV and therefore, are exposed to infection. In this context, the aim of our study is to design novel viral antigens in order to produce a more immunogenic vaccine against HBV in green plants, as a more cost-effective alternative. Our strategy is focused on the antigenic properties of the large (L) envelope protein and takes advantage of the ability of HBsAg to self-assemble into highly immunogenic subviral particles (SVPs), which are secreted and easier to purify. The HBsAg was used as a carrier of a highly antigenic L-derived epitope to generate two chimeric proteins: a) by inserting the pre-S1 21-47 peptide upstream the antigenic loop (AGL) of S and b) by replacing a fragment from AGL with the pre-S1-derived peptide, aiming to expose this new epitope at the SVP's surface. Expression of the chimeric proteins along with wild-type counterpart was investigated both in *Nicotiana benthamiana* and HEK-293T cells, as a reference system. The new antigens have similar folding and N-glycosylation patterns as the wild-type protein in both expression systems and are able to form similar SVPs, which were further purified by ultracentrifugation. The immunogenicity of these antigens and their ability to induce neutralizing antibodies are currently being evaluated in a small-animal model. To conclude, the novel antigens represent promising HBV vaccine candidates in order to develop a less expensive production system.

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POSTERS:

- ***PROTEIN SCIENCE***
- ***BIOCHEMISTRY AND MOLECULAR MEDICINE***
- ***NANOSTRUCTURES AND BIOMEDICAL APPLICATIONS***

PROTEIN SCIENCE

[P 1.1] **ROLE OF PROTEINS INVOLVED IN ERAD ON DIABETES MELLITUS**

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Type 2 diabetes mellitus is a chronic metabolic disease characterized by insulin resistance and chronic hyperglycemia that leads to deterioration of beta cell functions and reduction in pancreatic β-cells mass. Insulin is 10% of total protein synthesized under basal conditions, while under stimulated conditions biosynthesis of insulin can increase up to 50%. Almost half of newly synthesized proinsulin may fail to acquire its native form, and can be either refolded to their native structure or degraded throught ERAD (ER associated degradation) or autophagy. Accumulation of misfolded proinsulin in the endoplasmic reticulum (ER) leading to β-cell failure and T2DM. Our purpose was to investigate the role of proteins implicated in ERAD machinery, in insulin traffic, degradation and secretion in pancreatic β-cells and diabetic rats model.

We developed stable cell lines overexpressing proteins involved in ERAD and survey their effect on insulin synthesis and secretion. We found that overexpression of ERAD components increased glucose-stimulated insulin secretion in both first-phase and second-phase of insulin secretion. Treatment of cells with cycloheximide and pulse chase experiments showed that, overexpression of proteins involved in ERAD stabilizes insulin and increases insulin secretion, and western blot analysis revealed that under non-reducing conditions, decrease the folded intermediates and aggregates presented in the higher molecular. By testing the physiological role of proteins involved in ERAD, in diabetic rats we found increased serum insulin and decresed blood glucose levels suggesting regeneration of pancreatic β-cell. In conclusion, our data indicates that ERAD constituents have an essential role in insulin stability and secretion in β-cells being interesting targets for novel therapies aiming glucose lowering in T2DM.

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[P 1.2] SELECTION OF OIL-CONTAINING POPULATIONS OF PHOTOTROPHIC MICROORGANISMS FOR FURTHER BIODIESEL PRODUCTION

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In the last years there is an increasing interest in using phototrophic microorganisms, mainly green algae and cyanobacteria, for the conversion of their lipids to biodiesel. The main advantages of these microorganisms as compared with oil-rich seed are the followings:

i) higher oil content;
ii) higher rate of photosynthesis;
iii) higher biomass productivities;
iv) no need to compromise for the production of food, fodder and other products derived from crops (Chisti et al., 2007; Schenk et al., 2008; Zhu et al., 2014).

In this paper there are presented the results concerning the use of different inoculums for enrichment [both in BG_{11} medium and in BG_{11} supplemented with different quantities of bicarbonate (so called bicarbonate -based integrated carbon capture and algae production system –BICCAPS] and selection of oil-containing populations of phototrophic microorganisms, microalgae and cyanobacteria. Inoculums, from different sources, have been serially diluted (up to 10^{-12}) in 96 wheels microplate, at *p*H around 7 and under increasing values of *p*H (BICCAPS) ; for each wheel fluorescence signals, after Nile red addition, were measured . Nile red addition was done serially in time, in order to avoid a too high contribution of cells' natural fluorescence to the signal obtained. The populations (wheels) with highest signals, compared with positive control (olive oil) and negative control (pure water) where microscopically inspected (fluorescence in both red and green filters, as well as bright field images) and cultivated in Erlenmeyer flasks and further selected using the same protocol. Theses types of populations could be further used for lipid production first at the level of small pilot scale (10 L-100 L), and, after new selections and technological improvements, even at larger levels and different *p*H values (from 7.5 to 11.0).

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[P 1.3] MODULATION OF THE EXPRESSION OF PROTEIN TYROSINE PHOSPHATASES INVOLVED IN ENDOTHELIAL PROGENITOR CELLS DEVELOPMENT

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Endothelial progenitor cells (EPCs) are immature but lineage-committed angioblasts, derived from bone marrow-derived cells that play critical roles in adult vasculogenesis and endothelial homeostasis. They contribute to blood vessel formation and repair through their abilities to proliferate, differentiate into mature endothelial cells, integrate into the endothelial wall and produce proangiogenic cytokines. Therefore, in recent years EPCs have aroused considerable interest for different areas of therapeutic application including endothelial repair and neovascularization of ischemic organs.

To date, the molecular mechanisms that govern EPCs development and functions are poorly understood. Protein tyrosine phosphatases (PTPs) as key regulators of different signaling pathways might control EPC proliferation, differentiation as well as their vasculogenic potential. To better understand PTPs role in EPCs development, in the present study we investigated the influence of endothelial differentiation factors such as: collagen and endothelial growth factors (VEGF, IGF-1, EGF, bFGF) on the expression of five PTPs previously suggested to be involved in EPC development: PTPN11/SHP2, PTPRJ/DEP-1, PTPN1/PTP1B, PTPRM/PTPμ and PTPRB/VE-PTP. For this purpose, we estimated the mRNA and protein expression patterns of studied PTPs from EPCs grown in the following conditions: 1) medium with collagen and endothelial growth factors, 2) medium without collagen but with growth factors and 3) medium with collagen but without endothelial growth factors. Then, we compared the mRNA and protein expression levels with those found in mature endothelial cells. Finally, we observed notable differences in PTPs expression pattern in the three growth conditions and also when compared the expression of PTPs between the two cell lines. Our results revealed a significant influence of endothelial differentiation factors on the PTPs expression pattern that seems to be critical for EPC ability to proliferate and differentiate.

[P 1.4] IN VITRO AND IN SILICO STUDY OF INHIBITING ACTIVITY OF FULLERENOL ON $A\beta_{1-40}$ AMYLOID AGGREGATION

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Self-assembly of Aβ peptides and their accumulation in deposits in human brain is characteristic sign in Alzheimer's disease (AD). There is no cure for AD, but one of the promising approaches for AD treatment is an inhibition of amyloid aggregation [1].

We have investigated the ability of fullerenol to inhibit process of $A\beta_{40}$ amyloid fibrillization using *in vitro* and *in silico* methods. Fullerenol (F-OH), a water soluble derivative of fullerene C60 was prepared by Solvent-Free Reaction [2]. The inhibiting activity of fullerenol was investigated at four different $A\beta_{40}$: fullerenol ratios = 100:1, 20:1, 1:1 and 1:20 using Thioflavin T (ThT) assay and atomic force microscopy (AFM). The data have shown that interference of fullerenol with $A\beta_{40}$ led to decrease of ThT fluorescence intensities with increasing F-OH concentration. The lowest inhibitory activity (10%) was obtained for lowest concentration of fullerenol (ratio 100:1). The highest inhibitory activity (<70%) was observed for highest fullerenol concentration (ratio 1:20). In order to assess the toxicity of fullerenol we have measured its effect on viability of SH-SY5Y cell line by WST-1 assay. The 24 h exposure of cells to fullerenol caused no significant changes in viability relative to control at all studied concentrations.

The *in silico* data obtained by MM/PBSA Molecular mechanics Poisson–Boltzmann surface area (**MM-PBSA**) methods showed that fullerenol tightly binds to monomer $A\beta_{40}$. It was determined that for the fullerenol binding the electrostatic interactions are the most important; the key role in this binding plays polar negatively charged amino acids. The theoretical calculations thus, support the experimental data. In this work we have shown that fullerenol is able to prevent the formation of $A\beta_{40}$ peptide amyloid fibrils and can be a promising candidate in search of therapeutics for Alzheimer's disease.

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[P 1.5] INVESTIGATING AND ASSIGNING FUNCTIONS TO EDEM3 DOMAINS

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The ER (Endoplasmic reticulum) is the first step in protein folding and traffic to organelles of the secretory pathway, the plasma membrane or to the extracellular space. Nearly one-third of all newly synthesized proteins in mammalian cells are targeted to the ER for proper folding. Thus it contains high concentrations of molecular chaperons (calnexin, calreticulin, BiP), folding enzymes (glucosidases I and II, oxidoreductases) and quality control factors (ERManI, glucosyltransferase) to assist newly synthetized polypeptides to achieve native conformation. When properly folded, proteins are exported from the ER and transported to their final destination through the secretory pathway. Polypeptides that cannot achieve their native structure are recognized as aberrant products, retained in the ER and targeted for degradation. Through a series of tightly regulated processes called ER-associated protein degradation pathway (ERAD), misfolded proteins are retrotranslocated/dislocated to the cytosol for proteasomal degradation. EDEM 1, 2, 3 (ER-degradation enhancing alpha-mannosidase-like proteins) OS-9, XTP-3B and other ER resident proteins are likely to be involved in this process.

Our studies focus on EDEM3 protein and its role in ERAD. It has been reported that EDEM3 is a soluble member of G47 hydrolase family, located in the ER lumen of mammalian cells that accelerates ERAD of misfolded glycoproteins. We predicted the structure of EDEM3 domains using bioinformatics and biomolecular modeling to help unravel the function and role of EDEM3 and discover how possible mutations affect its function. Based on the 3D model of EDEM3 we constructed several mutants by deleting different domains predicted as important for different functions. Preliminary results confirm that EDEM3 and some of its mutants accelerate protein degradation and partly preserve the mannosidase activity. Further studies will be directed towards elucidating the role of each deleted domain in substrate binding or enzymatic activity.

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[P. 1.6] **FINE TUNING OF EPITOPE PRESENTATION BY KEY PLAYERS OF ERAD PATHWAY**

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Melanoma is the most aggressive form of skin cancer with very low lifespan and poor prognosis. The incidenence of melanoma has increased in the Western world over the last years [1], and thus an increase of new and efficient immunotherapies is required [2]. When melanoma arises, the immune system is capable to remove transformed cells by the action of cytotoxic T cells (CTL). For T cells to be activated antigenic peptides of melanoma cells in complex with MHC-I (major histocombatility complex) are exposed to the cell surface. One of the proteins processed to antigenic peptides in melanoma cells is tyrosinase, a differentiation antigen, biosynthesized and folded in the endoplasmic reticulum and upregulated in some melanomas. Properly folded tyrosinase is trafficked to melanosomes where the synthesis of melanin takes place; misfolded or partly folded tyrosinase molecules are redirected to proteasomal degradation through ERAD (Endoplasmic Reticulum Associated Degradation). In this way tyrosinase antigenic peptides are produced and presented to the cell surface.

One of the tyrosinase epitope presented at the cell surface is the 9 aminoacids sequence YMDGTMSQV (YMD), found in the protein sequence as YMNGTMSQV. Although the mechanism of ERAD is still under investigation, its main key-elements like EDEM proteins, OS9 , XTP and SEL1L were already described. Our aim is to investigate if overexpression of these components can modulate YMD presentation at the surface of melanoma cells. We overexpressed the main ERAD components in A375 amelanotic melanoma cell line stably expressing WT tyrosinase and measured the YMD peptide at the cell surface using high resolution mass spectrometry. Our results suggest that ERAD components modulate HLA-A2-mediated presentation of the YMD epitope, thus providing a platform for new therapies. Future experiments will focus on understanding the mechanisms by which ERAD can modulate the immune response in melanoma.

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[P 1.7] THE DEGRADATION OF MISFOLDED PROTEINS FROM THE ENDOPLASMIC RETICULUM IS MODULATED BY EDEM1

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Secretory and membrane proteins are translocated into the ER (endoplasmic reticulum), folded with the help of ER resident chaperones/enzymes and transported through the secretory pathway to target organelle. Misfolded or incompletely folded proteins may accumulate within the endoplasmic reticulum; to maintain homeostasis and prevent ER stress, disposal of misfolded proteins is mandatory and mainly occurs by ER associated protein degradation pathway (ERAD).

EDEM1 (ER degradation-enhancing α-mannosidase like protein 1) is involved in ERAD by recognizing terminally misfolded proteins that exit folding cycles and target them for dislocation form the ER. It accelerates the degradation of many ERAD substrates and delivers them to the adaptor protein SEL1L for retrotranslocation and proteasomal degradation. Our aim is to understand the molecular mechanism that discriminates native and misfolded proteins, with particular emphasis on EDEM1, and establish its role in ERAD and protein homeostasis.

We previously found that EDEM1 modulates the degradation of tyrosinase and its mutants, and that the N-terminal intrinsically disordered domain (IDD) of EDEM1 is required for its association with tyrosinase. Currently we extended our experiments to several other model ERAD substrates: alpha 1-antitrypsin and NHK mutant, Ri332 (truncated mutant) and a spliced variant of β-secretase (BACE-476).

Experimental results confirmed that association of client proteins with the IDD of EDEM1 is a general feature and key for their efficient disposal through ERAD and the mechanism identified for tyrosinase can be extended to other substrates. Future experiments will reveal if the interaction EDEM1- client proteins is direct and which domain(s) are involved in the interaction.

Acknowledgement. This work was supported by a CNCSIS grant, project number PN-II-ID-PCE-2012-4-0350

[P 1.8] **FOCUS ON CELL-PENETRATING PEPTIDES AND THEIR THERAPEUTIC APPLICATIONS**

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Cell-penetrating peptides (CPPs) are short peptides capable of crossing the plasma membranes of living cells, with or without carrying a cargo. CPPs are useful vectors which are able to deliver therapeutic agents (drug molecules, plasmid DNA, siRNA, imaging agents) both *in vitro* and *in vivo*.

CPPs are easy to obtain, characterize, and are adequate to deliver covalently or non-covalently conjugated bioactive cargoes (peptides, proteins, oligonucleotides, liposomes) inside cells in order to obtain high levels of gene expression, gene silencing, or tumor targeting. We developed a new strategy to obtain highly effective CPPs by chemically modifying the transportan 10 (TP10) sequence and therefore obtaining a new series of CPPs, named NickFect peptides. The design, peptide synthesis, mechanism of entrance into cells and the applications for the efficient delivery of therapeutic molecules such as plasmid DNA, SCO, siRNA into living cells mediated by NickFect peptides demonstrated the effectiveness and versatility of these delivery vectors and their potential to be further used in *in vivo* studies.

[P 1.9] THERMAL STABILITY AND FIBRILLIZATION OF LYSOZYME IN WATER-MISCIBLE IONIC LIQUIDS

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Ionic liquids (ILs) are widely used as novel solvents in many areas. The water miscible ILs can also serve as effective participants in various biological processes. For instance, ILs have been found to act as proper solvents for modulation of amyloid fibrilization process. The major advantage of ILs is that their physicochemical properties (density, viscosity, melting point, polarity, etc.) can be tuned by appropriate combination of cations and anions in order to obtain solvent with desired properties.

We have studied the effect of imidazolium-based ionic liquids with acetate and tetrafluoroborate as anions on thermal stability of lysozyme and morphology of amyloid fibrils using calorimetry, circular dichroism spectroscopy, AFM and computer image analysis. The reduction of transition temperature and enthalpy of lysozyme heat denaturation is observed at the presence of either ILs in concentration range 1-8% v/v in 2 mM glycine buffer, pH 2.7. All transitions are highly reversible, but slightly dependent on heating rate.

One of the important steps for amyloid fibrillization is destabilization of protein native state. Lysozyme at low protein concentration doesn't form fibrils at acidified water. Addition of either ILs leads to formation of lysozyme fibrils, with slightly slower kinetics of fibrillization at the presence of acetate comparing to tetrafluoroborate and with different morphology with distinct fibril types.

In this work we have shown that ionic liquids with imidazolium as cation and chaotropic anion tetrafluoroborate or kosmoptropic anion acetate decrease stability of lysozyme at acidic pH conditions with only slightly stronger effect of chaotropic ion suggesting more complicated mechanism than simplistic Hofmeister phenomena. Further research is needed for better understanding the effect of ILs on stability and self-assembly of proteins.

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[P 1.10] EPIGALLOCATECHIN-3-O-GALLATE REDUCES THE CLONOGENICITY, INDUCES THE MITOCONDRIAL DEPOLARIZATION AND INCREASES PRODUCTION OF REACTIVE OXYGEN SPECIES IN CANCER CELL LINES WITH ERBB PROTEINS OVEREXPRESSION

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The overexpression of ErbB proteins in cancer cells was associated with reduced apoptosis, increased proliferation and metastasis [1]. Epigalocatechin-3-O-gallate (EGCG), the main catechin from the green tea was shown to have anticancer properties in several tumor cell lines and in vivo models [2, 3]. We investigated the effect of EGCG on critical mediators of cell signaling in SK-BR-3 mammary and A-431 epidermoid cancer cell lines with high expression of ErbB2 and ErbB1, respectively. Flow cytometry data demonstrated that 48 h treatment with 50 μM EGCG induced partial inhibition of pAkt(S473), pERK(Y204) and pFAK(S910) in both cell lines. We further studied the clonogenic potential of EGCG treated A-431 and SK-BR-3 cells for 48 h, which demonstrated growth inhibition in both cell lines at lower concentrations (EC50 of 2.5 and 5.4 μM for A-431 and SK-BR-3, respectively). EGCG treatment for 72 h showed dose dependent increase in ROS and subsequent cell death. These observations were well correlated with the mitochondrial membrane depolarization induced by EGCG in the same treatment conditions: 72 h of incubation and concentrations ranging from 5 μM to 100 μM. Since the mitochondrial depolarization can be associated with both necrosis and apoptosis, we evaluated the nuclear staining which showed chromatin condensation and nuclear disassembly in both cell lines suggesting apoptosis as the probable mechanism of cell death. Our data suggests that EGCG therapy might reduce the unfavorable traits associated with ErbB proteins overexpression, partially inhibiting activated key mediators of the ErbB signaling pathways, reducing the colony forming ability and promoting cell death.

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[P 1.11] MOLECULAR CHAPERONES AND OTHER WAYS TO PREVENT IRREVERSIBLE AGGREGATION AND TO MAINTAIN PROTEIN STABILITY

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The molecular chaperones, present in all the compartments of the cell are mainly involved in protein folding but also in protein transport to IR or mitochondria, protection of partially folded protein against cellular stress, and help to degradation missfolded proteins. The previous main interest of our group was non enzymatic posttranslational modifications (PTM) of proteins, their mechanism, the consequences, as well as possible inhibition of the deleterious effects. It was known protein glycation is a long time reaction, but we demonstrated it inactivates the enzymes, in spite of their short life. Our collaboration with NLO,Oxford Univ. , inspired us to study the effect of the molecular chaperone ***alpha crystallin*** on protein modified by PTM, such as glycation, oxidation, aldehyde binding, carbamylation. Previous studies estimated ***α-crystallin*** chaperone activity by preventing heat induced-protein aggregation. We showed that ***α-crystallin*** specifically protects enzymes from inactivation by various PTM. Our data showed that chaperone mostly stabilized and protected enzyme from further denaturation, the enzyme refolding was less significant. The results indicated formation of chaperone-enzyme complex can explain mechanism of protection.

In addition to molecular chaperones, a number of small natural organic compounds such as sugars, amino acids, methylamines, under unfavorable environmental conditions, accumulate in cells, stabilize and protect proteins from stress. Our interest was directed to: 1) the protective effect of **SOS molecules** on enzymes under stress conditions (oxidation, glycation or chaotropic agents) 2) the effect of PTM (methylglyoxal and steroid binding) on alpha-crystallin chaperone activity and structure, and 3) protection from stress of alpha-crystallin and other lens proteins by SOS molecules. Trehalose, 6-aminohexanoic acid, and TMAO prevented glycation-induced inactivation of enzymes, at different protection level, suggesting different mechanisms could be involved. In addition, trehalose and 6-aminohexanoic acid completely recovered GuHCl- denatured G6PD; as far as we know renaturation of an enzyme was described for the first time. The modifying agents, MGO (a reactive glycation agent, present in human lens), and steroid binding induced lens proteins aggregation and lens opacification in lens organ culture, but trehalose had a protective effect; it also helped the recovery of GuHCl-denatured G6PD. The chaperone-like activity of naturally occuring SOS compounds need to be assessed carefully, as they seem to have a great potential for clinical use.

The correct folding /refolding of proteins became even more important when genetic engeneering methods developed and proteins started to be produced by recombinant DNA technology. For this reason, the **artificial chaperone** mecanism was discovered by scientists, inspired by naturally occuring chaperones.The protocol, generally consist of two steps, „*capturing-releasing*": a detergent binds to unfolded protein preventig aggregation, then a dextrin helps the protein to fold to its native state. Our data demonstrated that **the artificial chaperones** are able to support the refolding of large, complex proteins.

Bacterial molecular chaperone The bacterial molecular chaperones are important for the cell both under normal conditions, when there are present at low concentration in the cell, and under stress conditions such as inflammation or infection, when they accumulate to higher concentrations, helping the cell to survive the infectious process. In a recent and complex study on molecular chaperones in bacterial infections in the sheep we performed the first analysis of molecular chaperones GroEL and DnaK in pathogenic bacteria *B. ovis*, *S.* Abortus-ovis and *C. fetus* subsp. *Fetus.* The DnaK and GroEL molecular chaperones association with the antibioresistance in *S.* Abortusovis is an original aspect study, which contributes to understanding the complex mechanisms of the bacterial pathogenicity.

This abstract that outlines our scientific interest over more than twenty years should end in an optimistic way. **Chaperonotherapy,** a new hope for neurodegenerative diseases treatment?

[P 1.12] INFLUENCE OF HOFMEISTER ANIONS ON THERMAL STABILITY AND AMYLOID FIBRILS FORMATION OF LYSOZYME IN ACIDIC PH VALUE

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The Hofmeister series ranks the relative influence of ions on the physical behaviour of biomacromolecules, however effect of salt ions on protein amyloid fibrillization is unclear. We have explored an effect of various Hofmeister anions on stability and amyloid fibrillization of hen egg white lysozyme. The kinetic of fibril formation in presence of anions suggests that neutralization of positive surface charge of lysozyme due to interaction with anions accelerates lysozyme amyloid aggregation. The analysis of the conformational properties of formed fibrils shown that in presence of NaCl, lysozyme forms typical elongated fibrils with high content of Л-sheet. On the other hand, in the presence of both chaotropic perchlorate and kosmotropic sulfate anions the fibrils form clusters with secondary structure of Л-turn. Taken together, our study shows close correlation between Hofmeister effect of monovalent anions on lysozyme stability, their ability to accelerate nucleation phase of its fibrillization and consequently conformational properties of the formed fibrils.

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[P 1.13] THE *CAENORHABDITIS ELEGANS* EDEM-2 AFFECTS PROTEIN TRAFFIC AND LYSOSOMAL FUNCTION IN MULTIPLE CELL TYPES

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Proteasomal degradation and lysosomal degradation are two major protein degradative pathways essential for the maintenance of cellular homeostasis. The endoplasmic reticulum associated degradation (ERAD) eliminates toxic accumulation of misfolded or aggregated proteins from ER and is conserved in all multicellular organisms including *C. elegans*. EDEM-2 (ER Degradation Enhancing Manosidase-like-2) along with EDEM-1 and EDEM-3 belongs to the ER mannosidase-like family of proteins which in cell culture accelerate ER disposal and proteasomal degradation of misfolded proteins.

Here, we investigated the role of EDEM proteins in an intact organism and we show that they have an evolutionarily conserved function in ERAD. Moreover, knocking-out the ERAD components affects protein secretion in multiple cell types. Using various endocytic transport markers we found that in the absence of EDEM-2 there is a slight misrouting of lysosomal proteins, enlargement of the acidic compartments and a delay of lysosomal protein degradation. In conclusion, our results suggest that ERAD blockage has pleiotropic effects that affect cellular homeostasis.

Acknowledgement. This work was supported by CNCSIS grant PN-II-ID-PCE-2012-4-0350.

[P 1.14] IN SILICO STRUCTURAL INSIGHT ON MELANOGENIC PROTEINS INTERACTING WITH MEMBRANE COMPONENTS

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Introduction: DOPAchrome tautomerase (DCT) is a melanosomal protein involved in late steps of melanin production and mediator of antiapoptotic/ protective cellular pathways, which operates in melanocytic and non-melanocytic normal or transformed cells. However, no data are available about the cellular factors which control DCT fate in melanoma cells.

Previous experimental studies indicate colocalization of DCT with proteins clustered in cholesterol rich membranes, however the detailed interaction mechanism is still unknown.

Goal: To elucidate the structural factors responsible for the interaction between DCT transmembrane and C-terminal regions with different cholesterol rich membrane interactors and generate structural models consistent with available experimental data.

Results: Bioinformatic sequence analysis methods were used to delineate transmembrane domains from disordered regions in DCT and different protein interactors. Sequence pattern analysis revealed binding motifs in transmembrane DCT and specific patterns of hydrophobicity distribution in interactors subdomains respectively. Additionally, unstructured cytosolic domain of DCT display extended charge complementarity patterns with specific interactors.

Our theoretical data are strongly supported by experimental evidence confirming involvement of both transmembrane and cytosolic regions in the interaction between DCT and protein membrane components.

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[P 1.15] MASS SPECTROMETRY INTERACTION PROTEOMICS OF EDEM2 IN A MELANOMA CELL LINE

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The protein quality control is a mechanism by which the cell aims to reduce the load of misfolded proteins and prevent protein aggregation inside the endoplasmic reticulum, by sorting cargo proteins into correctly folded and amenable for the secretory pathway traffic, and into misfolded proteins that fail to reach their native state and are targeted for Endoplasmic-Reticulum Associated Degradation (ERAD) [1].The molecular mechanism by which the cell can target misfolded proteins for ERAD remains largely unknown, although important steps could be resolved by proteins like OS9, SEL1L, XTP3-B and EDEM (Endoplasmic reticulum Degradation-Enhancing alpha-Mannosidase-like) proteins: EDEM1, EDEM2 and EDEM3. It was previously shown that EDEM2 can actively participate in ERAD of misfolded substrates but its exact role still remains unknown. [2] Even more, most of the protein interactors partners were not described until now. The recent advances in technological development and in data analysis allow now to routine characterize protein-protein interactions using affinity purification combined with mass spectrometry (AP-MS).

We overexpressed EDEM2 with a human influenza hemagglutinin (HA) tag in the C-terminus of the protein in amelanotic melanoma A375 cells. Using AP-MS we aim to provide a comprehensive map of EDEM2 protein-protein interactions with a special attention on ERAD partners that could play a key role in targeting misfolded substrates to the ubiquitin proteasome machinery. Here we focus on different extraction conditions of protein-protein interactions relevant for EDEM 2 that can alter its protein interaction partners. As it was already reported for other proteins from ERAD pathway [3], we found that using digitonin we can recover the highest number of possible protein-protein interactions of EDEM2 with other ERAD participants. This was also confirmed by Western Blot experiments and by analyzing the total number of identified proteins in the AP-MS experiments with the three detergents tested (Digitonin, Triton X-100 and Hepes-Chaps), as we found an over 90 % increase in Digitonin compared with Triton and over 100 % increase in Digitonin compared with Hepes-Chaps. Future experiments will validate the new interaction partners in assessing a mechanism for ERAD substrates sorting.

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[P 1.16] **PRINCIPAL COMPONENT ANALYSIS OF AMINO ACIDS**

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Methods of multivariate analysis, namely principal component analysis (PCA) and cluster analysis (CA) were applied to classify 20 natural amino acids. We selected 18 characteristics, properties disposable from literature, as a basis for the classification. Such properties are molar mass, acid dissociation constants, isoelectric point, number of C, H, O, N and S atoms, Chou-Fasman parameters for predicting secondary structure in proteins, standard enthalpy of formation, standard state accessibility, average accessible surface area in proteins, solubility in water, hydrophobicity, and melting point.

The *Statistica* software package was used in the multivariate analysis of the variables (properties), and objects (amino acids). Significant correlations were found between these characteristics, confirming that the properties are related to each other and so the number of variables could be reduced. Loadings scatterplots are used as display tools for examining the relationships between properties, as well as dendrograms resulted from CA. For the classification of the amino acids, .the results of PCA are presented as scatterplots of scores, while dendrograms represent the outcome of cluster analysis.

They emphasize the similarities and differences between groups of amino acids. It is remarkable that this classification, based almost exclusively on structural features and physical properties is consistent with the usual “chemical” classifications based on the behavior of amino acids. It also accounts for the possibilities of mutual substitution of different amino acids in peptides and proteins.

The investigation performs a chemometrical characterization and classification of amino acids. This approach seems to be promising also for extension to other classes of biological relevant organic compounds.

[P 1.17] IDENTIFICATION OF INTERACTING PROTEINS WITH MELANOMA ANTIGEN DOPACHROME TAUTOMERASE BY MASS SPECTROMETRY ANALYSIS

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DOPAchrome tautomerase (DCT) or Tyrosinase Related Protein-2 is a melanoma antigen and a mediator of an anti-apoptotic / stress-resistance-tumor pathway. In this context the identification of the proteins interacting with DCT is of great significance for gaining more insights into the molecular mechanisms in which DCT is involved and possibly related to the initiation and perpetuation of this neoplasm. Moreover, as DCT is also a therapeutic target in malignant melanoma its protein interactors become also potential candidates for designing novel molecular anti-melanoma therapies. Mass spectrometry has emerged as a promising method in protein-protein interaction analysis as technological development of the instruments and of bioinformatics algorithms have increased in the last years.

Our goal was to investigate the DCT interactors in a melanoma cell line and correlate the identified molecules with additional information about the processes and mechanisms in which these are involved.

The cell lysate from a human melanoma cell line was subject to immunoaffinity enrichment using anti-DCT specific antibodies or a non-DCT antibody used as a negative control. The immunoprecipitated material was captured on Protein A-Sepharose and the eluted proteins were digested overnight with trypsin. The resulting peptides were further separated using nano LC coupled online with tandem mass spectrometry for peptide fragmentation. Analysis of the data revealed that ER-, ER-Golgi-, post-Golgi-proteins, transport proteins, proteins with antiapoptotic functions and involved in tumor progression, small GTPase regulators and proteins of the endocytic machineries were co-immunoprecipitated with the DCT-bait.

The most important DCT-interactors related to possible molecular mechanisms and pathways in malignant progression of melanoma are going to be taken into consideration for further functional studies.

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[P 1.18] **IDENTIFICATION OF ERAD COMPLEXES BY MASS SPECTROMETRY IN INS-1 PANCREATIC β AND HEK293T CELLS**

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Misfolded proteins are recognized and sent to degradation directly from the Endoplasmic Reticulum (ER) by a mechanism commonly known as the endoplasmic reticulum-associated degradation (ERAD) pathway, whilst properly folded proteins are allowed to leave the ER and are further trafficked through the secretory pathway. The accumulation of different glycoproteins incorrectly folded in the ER leads to several diseases including Parkinson's disease, diabetes or cystic fibrosis.

In order for proteins to be secreted or degraded they have to pass an quality control step where their folding state is assessed. One of the main protein in ERAD involved in recognizing and accelerating misfolded protein degradation, is considered to be EDEM1 (**E**R **D**egradation **E**nhancer alpha-**M**annosidase like 1). Along with EDEM2, EDEM3, OS-9, XTP3-B and other components of the ER dislocon EDEM1 is actively involved in selecting and guiding proteins for proteasomal degradation.

We present herein data related to ERAD complexes formed by EDEM1 in INS-1 pancreatic β-cells as compared to HEK293T, both overexpressing or not EDEM1. Endogenous or overexpressed EDEM1 was immunoprecipitated with specific antibodies and the co-immunoprecipitated proteins were identified by mass spectrometry (MS); a particular attention was payed to proteins involved in ERAD.

To understand the role of EDEM1 in ERAD in this process, we constructed a mutant lacking the intrinsically disordered domain that we have recently found to be involved in tyrosinase recognition within the ER. Both wild type and mutant EDEM1 were overexpressed in HEK293T cells and the isolated complexes were processed for MS analysis. We present here a comparative study of specific interactors of wild type and mutant EDEM1 involved in ERAD, as well as the interactors identified in INS1 cells.

EDEM1 association with the molecules involved in the recognition and dislocation folded proteins from RE in the cytosol is mainly due the presence of the intrinsically disordered domain of EDEM1 and less to the mannosidase like domain. Further studies will confirm the interactions detected by MS and establish the role of the complexes in ERAD.

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[P 1.19] **HYPOXIA MODULATES THE ENDOPLASMIC RETICULUM FUNCTION**

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Tumor cells use the unfolded protein response (UPR) as a mechanism of survival and hypoxia seems at least partialy responsible with the activation of UPR in cancer cells. Our goal was to find the trigger for UPR activation under hypoxia.

Therefore we studied maturation of a model secretory protein - immunoglobulin M (IgM) and found that IgM synthesis and secretion is disturbed under low oxygen concentration.

We investigated the redox state of several proteins that belong to the PDI family and used mass spectrometry to reveal the potential partners and substrates of several PDI-like proteins. PDI-like proteins appear to play a role in the retention of ER associated degradation substrates in anoxia.

Our current work aims to bring further details about PDI-like proteins role in health and disease.

[P 1.20] CHARACTERIZATION OF DOPACHROME TAUTOMERASE (DCT) –HIGH CLONES IN RELATION WITH MARKERS OF CELL PROLIFERATION AND SUBCELLULAR COMPARTMENTS

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A characteristic of malignant melanoma is the dowregulation of melanosomal antigens which are both tumor markers and therapeutic targets. DOPAchrome tautomerase (DCT) or Tyrosinase Related Protein -2 is a melanoma antigen, a target in vaccination therapy and a mediator of an antiapoptotic-/stress-resistance-tumor pathway. In a different study we have established that in a highly proliferative human melanoma cell line, during sub-confluent to confluent transition DCT expression is significantly increased and its subcellular distribution is severely altered.

The purpose of this study was to establish the characteristics of DCT-high cell population in relation with the main cellular compartments and processes.

The DCT expression was analyzed in the most proliferative cell subsets. A pool of melanoma cells were fluorescently labeled with carboxyfluorescein succinimidyl ester (CFSE), an agent which is incorpoated by cells and further distributed between daughter cells during proliferation. In this way the cell fluorescence is gradually diminishing in agreement with cell proliferation index and different cellular generations can be separated by flow cytometry and further anlyzed. The results showed that DCT expression is not significantly changed in cell populations with different proliferative capacity.

The experiments of co-localization between DCT and markers of cellular structures and compartments in high –DCT cell populations by immunoflorescent microscopy including, ER, Golgi, TGN, cytoskeleton, cell proliferations and apoptosis have been performed.

These data will guide the further approach of DCT-high subsets in relation with other processes associated with the metastatic progression.

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[P 1.21] ANTI-AMYLOID ACTIVITY OF 7-METHOXYTACRINE-ADAMANTYLAMINE HETERODIMERS IN THE TREATMENT OF ALZHEIMER'S DISEASE

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Colon cancer is a severe type of disease, in which surgical therapy complemented by radio- or chemotherapy, is hindered by the chemoresistance or secondary effect. Due to the complex and dynamic interactions in tumor microenvironment, there is constant need in designing new anti-cancer strategies that simultaneously target directly cancer cells development and indirectly the pro-tumor processes mediated by the crosstalk of cells in tumor millieu. The aim of our study was to investigate the cytotoxic actions of curcumin combined with 5-fluorouracil (5-FU) against C26 murine colon cancer cells co-cultured with macrophages and to elucidate the mechanisms underlying their effects. Curcumin, an anti-oxidant and anti-inflammatory agent, was administered prior to 5-FU treatment, to sensitize the cancer cells to this cytotoxic drug. The impact of these two agents on cell proliferation capacity, key oxidative stress, inflammatory, and angiogenic markers was evaluated, under normoxic or hypoxic culture conditions. Our preliminary data revealed that, after 48 h of treatment, curcumin (17 μM) combined with 5-FU (2 μM) inhibits C26 cancer cell proliferation (>80% compared to control) in both monoculture and co-culture system, in normoxia, as shown by BrdU incorporation assay. This inhibitory effect was maintained in hypoxic conditions, but only with the use of higher concentrations of curcumin (100 μM), in both monoculture or co-culture systems. No further improvement of these cytotoxic effects were seen when combined *vs.* individual administration of these agents was applied. Further experiments should address the role of macrophages in orchestrating these anti-tumor actions of curcumin and 5-FU. Our results may offer detailed information on how the tumor-associated processes emerging from the malignant crosstalk of macrophages and colon cancer cells can be modulated for future anti-cancer therapeutic approaches.

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[P 1.22] **ENDOFERTIL- A MOLECULAR THERAPEUTICAL STRATEGY FOR ENDOMETRIOSIS**

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Endometriosis is a benign condition of the female reproductive tract characterized by the implantation of endometrial tissue outside the uterine cavity. The socio-economic burden of the disease is impressive: impairment of work capacity, sick leave, high costs of treatments and the frequent use of assisted reproductive techniques to achieve pregnancy lead to a diminished quality of life for many women.

EndoFertil project aims to shed light on possible ways to modify the current staging and prognosis protocols with regard to the molecular and cellular abnormalities encountered in this disease. Moreover, the assessment of hormone receptor expression in women with endometriosis could help guiding the therapeutic use of specific drugs.

In a first step of the project we have set up a battery of parameters and protocols for analysis of normal and ectopic implant tissue from patients undergoing hysterectomy versus those with endometriosis, respectively. Using immunoblotting and fluorescence-based techniques we approached the assessment of immune status and invasive potential of donor specimens. Comparative western blotting analysis between normal and diseased patterns of cadherins expression was performed. Using flow cytometry the subsets of leukocytes present in the endometrium and implants were quantified. In parallel, the stromal fraction was identified using a cocktail of specific markers (CD13, CD29, CD90) as indicator of mesenchymal stem cells presence. A method of isolation and culture for possible circulating stem cells in mestrual blood is also presented that will allow the research on their potential role in endometriosis ethiology.

Based on the corroboration between analytical determinations and clinical evaluation of the donor patients a strategy for a possible near future therapeutical approach will be established.

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[P 1.23] UNVEILING THE FUNCTIONS OF DOPACHROME TAUTOMERASE IN MELANOMA CELLS

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Dopachrome tautomerase (Dct), an enzyme mostly known for its role in melanin biosynthesis in melanocytes and retinal pigment epithelium, appears to also play other essential functions in some tissues. Thus, in mouse neuronal progenitor cells Dct is required for cell proliferation. In melanoma and glioblastoma cells Dct confers resistance to DNA damage drugs such as carboplatin and methotrexate. Recently, it was found that the *Caenorhabditis elegans* Dct homolog is secreted by ASJ sensory neurons and taken up by the germ cells, where it downregulates the p53 protein levels and blocks the apoptosis. Moreover, in some human melanoma cell lines Dct seems to counteract the apoptosis, possibly through its L-dopachrome tautomerase activity and interfering with the p53 apoptotic pathway.

p53 is a key transcription factor which controls cell cycle arrest, DNA repair, apoptosis, senescence, and autophagy pathways, under various genetic, environmental, and metabolic stimuli. In DNA damaging conditions p53 is the switch that cells use to decide whether they can survive and repair their damage or induce their apoptosis in order to prevent the body from maintaining deleterious cells. The activity and intracellular localization of this essential molecular switch are regulated by the cells through complex integration of numerous post-translational modifications (e.g., phosphorylation, ubiquitination, and acetylation).

This study presents preliminary data on the mechanisms through which Dct promotes the proliferation of melanoma cells, looking in particular at the upstream regulators and downstream effectors of p53. We found that the depletion of Trp2 led to a stabilization of p53 protein and did not interfere with its translocation into the nucleus. Interestingly, the main regulator of p53 stability, Mdm2 ubiquitin ligase, was also more stable in cells depleted of Dct. The analysis of some downstream effectors suggests that the depletion of Dct leads to a cell cycle arrest in MJS cells.

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[P 1.24] **DnaK AND GroEL MOLECULAR CHAPERONES ARE REQUIRED FOR THE ADAPTATIVE TOLERANCE RESPONSE IN *SALMONELLA* ABORTUSOVIS**

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Salmonella Abortusovis is a pathogen with host-restriction which affect the reproductive tract of sheep implying some adaptative pathogenic mechanisms specialized for the survival and dissemination of the bacteria under hostile conditions from the host. The role of molecular chaperones in *Salmonella* adaptative tolerance response has been previously mentioned, but nothing was published on *S.* Abortusovis in this respect, as far as we know.

In the present work, in order to understand any possible contribution of the molecular chaperones DnaK and GroEL in *S.* Abortusovis adaptative tolerance response, the effect of adaptation to mild stress conditions on the survival of *S.* Abortusovis in the extreme conditions was analysed and the molecular chaperones expression was quantified at the transcriptional and translational levels. The survival tests showed that the adaptation of *S.* Abortusovis exposed to various stress conditions induce the cross-resistance at high temperatures, antibiotics, extreme oxidative and acidic conditions.

These results were consistent with the high level of DnaK and GroEL expression analysed by quantitative real time RT-PCR method and Western blot analysis.

Here we demonstrate the correlation between the increasing bacterial resistance after the adaptation to mild stress conditions and the molecular chaperones, their expression under stress conditions suggesting the chaperones role in the adaptative tolerance response in *S.* Abortusovis.

BIOCHEMISTRY AND MOLECULAR MEDICINE

[P 2.1] DIRECT DETECTION OF FREE RADICALS IN POLYPHENOLS AND CORRELATION WITH ANTIOXIDANT CAPACITY

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The polyphenolic content and to some extent details about individual components can be detected in EPR spectra of natural extracts treated with a base, where free radical signal can be detected. A direct relation between the intensity of the signal and the content of phenols was previously observed in propolis extracts using similar experiments [1]. The mechanism of radical formation has not been elucidated yet in detail, but it is expected to be based on the generation of semiquinone anion radical during the autooxidation of polyphenolic compounds treated with alkali compound in the presence of molecular oxygen [2]. Probing the phenolic content of a natural extract by such alkaline treatments, EPR spectra would have the advantages of directly detecting free radicals pertaining to the sample (as opposed to indirectly detecting them via reaction with ABTS, DPPH, etc) including structural information (as each radical is expected to display a different EPR lineshape) – while also allowing for the kinetic approaches.

Here, the mechanisms of free radical formation are explored for pure polyphenols as well as for some natural extracts (*Hedera*, *Lamiaceae* family) trying to establish a link between EPR parameters and antioxidant capacity determined by different methods (DPPH, inhibition of peroxidase activity of hemoglobin, etc).

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[P 2.2] LINEAGE SPECIFIC EVOLUTION OF THE VNTR COMPOSITE RETROTRANSPOSON CENTRAL DOMAIN AND ITS ROLE IN RETROTRANSPOSITION OF GIBBON LAVA ELEMENTS

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Retrotransposons are a class of mobile genetic elements that use retroviral-like reverse transcription during transposition. The consequences of retrotransposon-induced genomic alterations include changes leading to interspecific and interindividual differences as well as a broad spectrum of diseases.

VNTR composite retrotransposons - SVA, LAVA, PVA and FVA - are specific to hominoid primates. Their assembly, the evolution of their 5' and 3' domains as well as the functional significance of the shared 5' Alu-like region are well understood. The central VNTR domain - long assumed to represent a random collection of 30-50bp GC-rich repeats - has recently attracted attention in the context of regulation of SVA expression. Based on differences observed in the VNTR between SVA and LAVA as well as between LAVA subfamilies, we initiated a detailed analysis of their VNTR domains across hominoid primates. An initial inventory identified 19 repeat unit (RU) types - only three of them shared between SVA and LAVA. Later analysis revealed that VNTR organization is non-random with conserved RU arrays present at both its 5' and 3' end. Furthermore, the younger SVA subfamilies are characterized by highly organized internal RU arrays. The composition of these arrays is specific to the human/chimpanzee and orangutan lineages, respectively. A microhomology-driven mechanism was found to mediate expansion/contraction of the VNTR domain at the DNA level.

Elements of all four VNTR composite families have been shown to be mobilized by the autonomous LINE1 retrotransposon in *trans*. The key determinants of SVA mobilization are found in the 5' hexameric repeat/Alu-like region. We now demonstrate that in LAVA, by contrast, the VNTR domain determines mobilization efficiency in the context of domain swaps between active and inactive elements.

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[P 2.3] THE CHARACTERISATION OF SOME BIOACTIVE COMPOUNDS IN DIFFERENT *ALOE* SPECIES

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Among all the *Aloe* species cultivated worldwide, *Aloe barbadensis* Miller L. and *Aloe arborescens* L. are the most common but various other species are known. Aloe species are used in traditional medicine due to their therapeutic effects such as: antimicrobial, anti-inflammatory, antitumor and antioxidant activity. However, the biological activities of the plant should be attributed to a synergistic action of various compounds rather than to one single class of compounds. The strong therapeutic properties were demonstrated especially for *Aloe vera*, *Aloe arborescens* or *Aloe ferox* species. The plant is characterized by big, pulpy, succulent leaves, rich in different bioactive compounds, including carotenoids. The analysis were carried out on seven different *Aloe* species. The purpose of this study is to set the antioxidant activity of different Aloe species in relation with the content in the most important antioxidants: ascorbic acid, carotenoids and tocopherols. Antioxidants were determined by volumetric (vitamin C), spectrophotometric and chromatographic HPLC/PDA/FL (carotenoids and tocopherols) methods. The stable radical DPPH test and the ORAC assay were used in order to determine the *in vitro* radical scavenging capacity of plant samples. Lutein and beta-caroten were the main carotenoid identified in all samples.

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[P 2.4] THE INCLUSION OF GRAPE POMACE IN WEANING PIG DIET INFLUENCES THE HUMORAL IMMUNE RESPONSE

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The aim of the present study was to evaluate the influence of two diets containing 3% grape pomace (GP) with seed (TN1/GPS+) or without (TP2/GPS-) on the humoral immune response on weaning piglets. The polyphenols composition of the two grape pomaces were evaluated by LC-MS before being incorporated in the compound feed. 18 crossbred starter piglets, were divided into 3 experimental groups (6 piglets/group) and assigned to one of the 3 treatments: control (normal diet for weaned pig-C); diet with integral GP (diet 1-D1); diet with GP without seeds (diet 2-D2) for 42 days. At the end of this period, blood samples were collected from all three groups and kept at -80^{0}C for further analysis.

The main polyphenolic compounds tentatively identified from the aqueous extract of GPs were: gallic acid, (epi)catechin, procyanidin and derivatives of phenolic acids like, protocatechuic, caffeic, gallic and vanilic.

The nonspecific immunoglobulins A, M and G (IgA IgM and IgG) measurement (by ELISA) showed that GP diets modulated the humoral immune response of the piglets. GPS- induced the increasing of the synthesis IgM and IgG with key role in the generation of the first and long lasting immune response, while GPS+ had a contrary effect.

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[P 2. 5] ANTIOXIDANT EFFECTS OF SEA BUCKTHORN FRUIT IN HIGH FAT DIET RAT MODEL

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The Sea Buckthorn fruit is rich in flavonols, carotenoids, palmitoleic acid, tocopherols, vitamin C and phytosterols and has anti-inflammatory and antioxidant properties. The flavonoids, quercetin and isorhamnetin from the Sea Buckthorn fruit have different tissue distribution.

The aim of this study is to analyze the antioxidant effects of the Sea Buckthorn fruit on different tissues: brain, liver, pancreas, spleen, kidney and heart in an obesity mouse model.

Wistar rats (n=20) were raised for two months on a high caloric and high fat diet and they became obese. Then, they were divided in four groups, each group with 5 rats and they continued with different diets with or without Sea buckthorn fruit as a supplement, 2g/rat/day, for the next two months. Group1 continued the high fat diet without supplements, group2 continued the high fat diet with supplement, group3 continued with standard diet without supplement and group 4 had standard diet with supplement. Plasma variables and total glutathione and total thiols from tissue homogenates were measured by spectrophotometry.

In each tissue the lowest values for the antioxidant parameters and the worst metabolic profile was observed in the group1. A significant increase of total thiols and glutathione was observed in group 4, in liver ($p<0.05$) and in pancreas ($p<0.03$). Also, a significant decrease in plasma triglycerides ($p<0.05$) and plasma glucose ($p<0.02$) in group 4 versus group1 was measured.

In conclusion, in obese Wistar rats, the changing of the fat diet with a standard diet associated with Sea buckthorn fruit, for at least 2 months, improved the antioxidant defence in the pancreas and in the liver and reduce glycemia and triglyceridemia. The comparison of the antioxidant potential of the sea buckthorn on different tissues: brain, liver, pancreas, spleen, kidney and heart shows that the best antioxidant effect is on the liver and the pancreas.

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[P 2.6] THE PROFILE OF THE SECONDARY METABOLIC COMPOUNDS FROM *LYCOPERSICON ESCULENTUM* PLANTS IN STRESS CONDITIONS

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Tomato (*Solanum lycopersicum* L.) is an annual plant native to South America with worldwide enormous economic value. Environmental stresses, such as high or low temperature, drought, and high ozone concentration have large effects on tomato physiological activity, significantly reducing the productivity. *Lycopersicon esculentum* plants were used to test different abiotic stresses: drought, heat, cold and ozone.

The emission of a wide array of mono (2-carene, alpha and beta pinene, limonene, alpha and beta phelandrene, beta-ocimene) and sesquiterpene (beta and delta elemene, beta-caryophylene and alpha-humulene) were detected. In case of cold and heat stresses of *Lycopersicon esculentum* the emission of some monoterpene and beta-caryophyllene is increasing with the strength of the stress. β-phellandrene and β-ocimene emission rates are increasing exponential with decreasing in temperature for cold stress and increasing for heat stress.

In case of drought stress, if in the first days of lack of water the monoterpene emission is increasing, in the following days the monoterpene emission is decreasing and sesquiterpene emission is increasing. In addition, our results show very high terpene emission rates just after the re-watering of plants.

The results from DNA extraction showed that fragmentation happens in DNA molecule of tomato after heating, especially when they are kept at room temperature more than 24 hours.

[P 2.7] IS SIALIC ACID A PREDICTIVE MARKER FOR DIABETIC NEUROPATHY?

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Sialic acid is a marker of the acute-phase response related to the presence of diabetic microvascular complication. The objective of this study was to demonstrate if increased sialic acid concentration can help in predicting the occurrence of microvascular complication such diabetic nephropathy.

We investigated the serum concentration of sialic acid, glucose and creatinine in 34 type-2 diabetics and 10 healthy subjects (controls) in which were used criteria based on gender and age. The aim of the study was explained to the subjects and those who gave their consent were included in it.

There was a significantly increasing trend of serum sialic acid ($p < 0.001$), glucose ($p < 0.001$) and creatinine ($p < 0.01$) levels in diabetics compared to control subjects for both studied genders. We also observed a progressive increase in all three biomarkers concentration as age advanced. Significant positive correlation was found between sialic acid and creatinine concentrations: the identified correlation coefficient $R = 0.652$ has an associated probability $p < 0.001 < \alpha = 0.05$.

Considering that sialic acid is an important component of vascular cell membrane, we can relate its increased concentration to extensive microvascular damage in type 2 diabetes patients. Patient medical history and estimation of sialic acid levels can facilitate the early prediction and prevention of microvascular complications of type 2 diabetes like nephropathy, retinopathy and cardiovascular disease.

[P 2.8] COMPUTATIONAL INSIGHT INTO THE STRUCTURE, DYNAMICS AND INTERACTIONS OF THE HUMAN SEROTONIN TRANSPORTER

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Serotonin transporter (SERT) is an essential drug target used in the treatment of depression and pain, but also other medical conditions including ADHD or irritable bowel syndrome. This protein contains an intrinsically-disordered N-terminal domain, a transmembrane domain and a C-terminal domain. The transmembrane domain is the actual drug receptor and has been extensively studied by various in vivo and in vitro methods, however the structure of human SERT was not elucidated yet, which seriously hampered the design of more effective antidepressants.

Using an original modeling procedure tailored to membrane-embedded proteins, we generated a model of human SERT, based on high homology with recent x-ray structures of dopamine transporter and LeuT, a bacterial SERT homolog, in complex with sertraline, R- and S-fluoxetine. Our structural model allowed identification of human SERT regions critical for drug binding, opening important perspectives for structure-based drug design of more effective ligands. On the other hand, analysis of the N-terminal domain is more challenging due to its intrinsic disordered nature, therefore the conformational transitions of this region and and its contribution to function modulation are poorly understood.

In this regard, we used a combination of ab-initio modeling and molecular dynamics (MD) simulations to evaluate the conformational changes of the N-terminal domain. We generated an ab-initio structural model which was fully hydrated and ionized, resulting a simulation system of over 70,000 atoms. This system was further subject to MD simulations during 60ns. Our MD simulations identified the presence of a short stable helical segment forming a potential folding nucleation site and a more flexible beta-hairpin structural motif which is possibly involved in other protein-protein interactions.

We also identified numerous phosphorilation sites in the region, which could indicate a complex mechanism governing the interaction of this N-terminal domain with the membrane and modulating the human SERT physiological function.

[P 2.9] DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS INTO KERATINOCYTE-LIKE CELLS IN 2D AND 3D CULTURE SYSTEMS

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Nowadays, an alternative treatment for skin repair is represented by mesenchymal stem cells, due to their high proliferation rate *in vitro* and multipotency. In the present study, human adipose-derived stem cells (hASC) were isolated from lipoaspirate, their immunophenotypic characteristics were evaluated by flow cytometry. Their differentiation potential towards keratinocytes was investigated after cells cultivation on a 2D surface (tissue culture plate) and on a 3D culture system (collagen based scaffold), in a specific induction medium, for 21 days. Their cell morphology was observed by light and electron microscopy and keratinocytes specific gene expression was revealed using RT-PCR.

The results obtained showed that the cells isolated from adipose tissue presented spindle-shaped morphology, expressed the mesenchymal stem cells specific markers, CD 73, CD 90, CD 105 and were negative for hematopoietic stem cell marker CD34. After cell cultivation in specific adipogenic and osteogenic media, they were differentiated into adipocyte and osteoblast lineages.In the presence of keratinocyte induction medium, differentiated cells underwent changes in cell morphology, having a polygonal appearance, after three weeks of cultivation in both 2D and 3D systems. In addition, RT-PCR results showed that the cells were positive for cytokeratin 19 and involucrin, demonstrating that hASC were induced towards the keratinocyte lineage.

In conclusion, this study demonstrates that hASC can be differentiated in vitro to keratinocyte-like cells in both 2D and 3D culture systems, under specific induction media exhibiting great potential in cell-based therapies for skin wound healing.

[P 2.10] STEM CELLS DERIVED FROM AMNIOTIC FLUID: MOLECULAR AND ELECTROPHISIOLOGICAL CHARACTERIZATION

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Human amniotic fluid cells holds a therapeutic potential for human diseases, as different populations of amniotic fluid stem cells (AFSC) have been isolated from amniotic fluid. They represent a novel class of pluripotent stem cells with intermediate characteristics between embryonic and adult stem cells. They are able to differentiate into lineages representative of all three germ layers and without *in vivo* tumorigenicity. We characterized human AFSC using whole-cell patch clamp, flow cytometry and gene expression RT-PCR array profiling for human stem cell genes. Our results show five ion current components (delayed rectifier, A-type, inward rectifier, big conductance Ca^{2+}-dependent K^+ currents, fast voltage-dependent Na^+ currents). Senescent AFSC showed reduced expression of CD44, CD90, CD133, marked up-regulation of interferon gamma and telomerase reverse transcriptase genes (over 500-fold increase), increased cycle-dependent kinase 4 inhibitors, p53-binding protein 1, and decreased calreticulin and CD44. HLA-ABC immune expression was similar, and HLA-DR expression very low in both cell types. An interesting subset of cryopreserved AFSC (approximately 1/3 of them) featured elements suggestive of neuronal differentiation: large inward rectifier K^+ currents and voltage-dependent Na^+ currents. In all AFSC cells, in both culture conditions, at patch rupture the K^+ currents were very low, and they increased progressively over several minutes upon cytoplasm dialysis with pipette solution, containing high amounts of calcium buffer, as an effect of direct and possibly indirect modulation of K^+ channels by internal calcium via calcium sensing proteins. Using a combination of biophysical and molecular biology methods we have succeeded to render a detailed characterization of the dominant electrophysiology, immunophenotype and gene expression features of human AFSC that will contribute to identifying ion channels present in stem cells and their role during cell growth in culture in corelation with cells immunophenotype.

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[P 2.11] CHANGES INDUCED BY *AMINOGLYCOSIDES* AT MEMBRANE LEVEL

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Aminoglycoside antibiotics area therapeutically important family and are used to treat patients with serious infections caused by Gram negative as well as patients suffering by febrile neutropenia. Because these antibiotics are used in various long term treatments and because the membrane is the first barrier involved in antibiotics efficacy, the study of the interaction between drug and membrane (artificial or natural) is important for understanding the mechanism of this interaction.

Our aim was to study the modification in the membrane organization induced by aminoglycosides (gentamicin, amikacin and kanamycin).

We tested the effect of 3 aminoglycosides: gentamicin, amikacin and kanamycin on artificial membranes (DMPC liposomes and DMPC mixed with cardiolipin) and membranes of EA.hy926 human endothelial cells. We used laurdan fluorescence spectroscopy and fluorescence anisotropy.

In case of the DMPC liposome membrane, the presence of aminoglycoside antibiotics does not change the generalized polarization (GP) values at all tested temperatures.In the case of liposomes in which the DMPC was mixed with cardiolipin, the presence of antibiotics induced changes in membrane general polarization (GP). The effect was most pronounced in case of gentamicin, being statistically significant at almost all tested temperatures whereas amikacin and kanamycin effect was significant only on specific temperature domains. In case of EA cells, the antibiotics induced a GP increase at all temperatures, the most pronounced effect being that of gentamicin. When membrane fluorescence anisotropy was measured, all three aminoglycosides (gentamicin, kanamycin, amikacin) induced a rigidityof liposomal membrane at all tested temperatures (15-37°C). The rigidity of liposomal membrane in the presence of aminoglycosides occurred also when mixed composition membranes (DMPC + CL) were used. The magnitude of the effect decreased in *gentamicin-amikacin-kanamycin* order. All three aminoglycosides caused also the rigidity increase of the EA cell membrane, the effect being more pronounced at temperatures exceeding 27°C.

[P 2.12] BLOOD BIOMARKERS OF PROSTATE DISEASES, AS IDENTIFIED BY LC-MS LIPIDOMICS

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The omics' profile of body biofluids can reflect the general condition of the human organism and indicate the presence of different pathologies, such as inflamations (hyperplasia) or cancer, in different stages. Detection of molecular biomarkers by qualitative fingerprints and quantitative profiling is well established in proteomics (mainly based on electrophoresis) and has a great potential by metabolomics technology based on gas- or liquid- chromatography coupled with mass spectrometry detection. For example, Prostate Specific Antigen (PSA) is well known as a proteomic biomarker used for diagnosis of prostate cancer, but has a low grade prediction, nowadays the small lipid metabolites being expected to be the best biomarkers of diagnosis and prognosis[1].

The aim of this study is to realize an adequate LC-MS fingerprinting and quantitative profiling of lipids and selection, by biostatistical techniques of specific biomarkers[2,3]. Blood samples, originating from healthy men (M) and patients with prostate benign hyperplasia (H), presumed cancer (samples collected before biopsy - CB), confirmed cancer (samples collected before surgery - CO) were processed by a preliminary precipitation of proteins and solvent extraction of lipids (Bligh & Dyer method) which were subjected to HPLC–ESI(+)QTOF-MS measurements. The control of the instrument and data processing was done using TofControl 3.2 and Data Analysis 4.2 (BrukerDaltonics). To process the raw matrix data, Profile Analysis 2.0 software from Bruker was applied for alignment and advance bucketing and then, the multivariate analysis (PCA and Cluster Analysis) by Unscrambler 10.1 software.

The untargeted biostatistical processing of comparative chromatograms, using peak areas and m/z values, suggested that molecules with m/z values of 316.3227 (eicosanoic acid or decanoylcarnitine), 369.3528 (tetracosanoic acid or 6 keto prostaglandin E1 or 5,12 diHPETE isomers), 415.2118 (ascorbyl palmitate), 432.240 (N-stearoyl phenylalanine) as well diacylglicerols, phosphatydylcholines, phosphatidylserines and ceramides can represent good diagnosis biomarkers, which can evidentate qualitative differences between the control and pathologic groups (as revealed by PCA analysis).

Further studies are needed to confirm the quantitative variations of such biomarkers, individually or related to the metabolic chain, for normal versus cancer patients. Such lipidomic biomarkers can be useful for an accurate diagnosis, prognosis and clinical decisions for furthertreatmenst of prostate cancer.

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[P 2.13] HIF-1 A –THE KEY PLAYER IN THE SIMVASTATIN CYTOTOXICITY ON B16.F10 MURINE MELANOMA CELLS

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To survive in the hypoxic microenvironment characteristic to all solid tumors exceeding 2mm^3, tumor cells express different growth factors that mainly promote angiogenesis, cell proliferation, and metastasis. A transcription factor with pivotal role in regulating the adaptive response of cancer cells to hypoxia is hypoxia inducible factor-1α (HIF-1α). Moreover, our previous studies demonstrated that simvastatin (SIM) strongly inhibited the proliferation and metastatic capacity of B16.F10 murine melanoma cells mainly via the inhibition of constitutive expression of HIF-1α (Alupei et al., 2014). Therefore, the present study aims to investigate the cytotoxicity of SIM on B16.F10 melanoma cells under hypoxic conditions in tight connection with the statin inhibitory effects on the production of HIF-1α in these cancer cells. Our results have shown that SIM induced a strong inhibition of tumor cell proliferation via the strong suppression of HIF-1α. Moreover, under hypoxic conditions SIM drastically decreased angiogenic and inflammatory capacity of B16.F10 cancer cells.

Thus, the cytotoxicity of SIM on melanoma cells involves the suppression of HIF-1α production in these cells. The consequences of this effect are dependent on the role of this molecule under different tumor microenvironment conditions. Therefore, SIM might be a promising therapeutic agent for melanoma treatment.

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[P 2.14] POSITIVE ASSOCIATION BETWEEN PROINFLAMMATORY CYTOKINES AND TOTAL OXIDANT STATUS IN TYPE 2 DIABETES MELLITUS PATIENTS WITH ELEVATED HOMA-IR

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Pathogenic effects of high glucose are mediated by reactive oxygen species which elicits systemic inflammation, accelerating the progression of type 2 diabetes (T2D). The aim of this study was to study the proinflammatory cytokines in T2D patients divided according to the severity of insulin resistance.

118 T2D patients were divided in three groups according to the HOMA-IR (Homeostatic Model Assessment Estimated Insulin Resistance) values: ≤3(1), between 3 and 5(2) and ≥5(3). Anthropometric measurements, routine blood tests, total oxidant status (TOS), total antioxidant status (TAS) and ELISA measurement for TNF-α, IL-6, leptin, insulin, C peptide and proinsulin were included. Respiratory burst (RB) of isolated peripheral blood mononuclear cells (PBMC) was measured by chemiluminescence. In group 2 versus 1: RB, TNF-α and uric acid were significantly higher while in group 3 versus 1: RB, TNF-α, IL-6, leptin, proinsulin, C peptide and TOS were higher. In our study the inflammatory cytokines, IL-6 and TNF-α were associated with HOMA-IR, proinsulin level and with the ratio proinsulin/insulin. These values indicate a compensatory effect over functioning of beta cells to nullify the insulin resistance produced by TNF-α.

Our study highlights that T2D patients with HOMA-IR higher than 3 had also increased levels of proinflammatory cytokines, TOS and RB.

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[P 2.15] NADPH-OXIDASE – KEY-PLAYER IN MAINTAINING THE OXIDATIVE STRESS INDUCED BY TUMOR-ASSOCIATED MACROPHAGES CO-CULTIVATED WITH C26 MURINE COLON CARCINOMA CELLS

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Oxidative stress generated by tumor-associated macrophages (TAMs) plays an important role in promoting angiogenesis, inflamation, and cell proliferation. The physiological range of reactive oxygen species (ROS) necessary for tumor cell proliferation is mainly maintained via the activity of macrophage NADPH-oxidase. Therefore the goal of our study was to evaluate the influence of this enzyme of TAMs on the proliferation, angiogenic, and inflammatory capacity of C26 murine colon carcinoma cells.

To this purpose we incubated a mixt culture of C26 murine colon carcinoma cells and intraperitoneal murine macrophages at a density ratio of 4:1 with 300μM apocynin (inhibits the assembly of a functional NADPH-oxidase). After 48 h of treatment we evaluated the proliferative capacity of tumor cells via BrdU immunoassay, expression levels of the NF-kB by Western Blot analysis, and the production of angiogenic/inflammatory proteins via a protein array. To assess the antioxidant effect of apocynin we quantified malondialdehyde (MDA) via HPLC.

Our results have shown that inhibition of NADPH-oxidase lowered the proliferation rate of C26 cells co-cultured with macrophages by 29% compared to the proliferation of the same cells in co-culture which were not treated with apocynin. No effects were observed on the expression level of NF-kB after inhibition of NADPH-oxidase. Nevertheless, incubation with apocynin notably reduced the expression of most of the angiogenic and inflammatory proteins as well as the level of MDA in co-culture cell lysates. Altogether these data suggest that suppression of oxidative stress via NADPH-oxidase inhibition decreased the levels of angiogenic and inflammatory proteins in co-culture cell lysates.

In conclusion, the oxidative stress generated by TAMs is a key-player in tumor progression by stimulating cellular processes like angiogenesis and inflammation.

Acknowledgements. This work was supported by UEFISCDI (Romanian Ministry of Education, Research and Innovation) project PN-II-PTPCCA-2011-3-2-1060, contract nr.95/2012 and the strategic grant POSDRU/187/1.5/S/155383.

[P 2.16] SYNERGISTIC EFFECT OF LIPOSOMAL 5-FLUOROURACIL AND PREDNISOLONE IN COLORECTAL CANCER TREATMENT

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Colon cancer is a severe type of disease, in which surgical therapy complemented by radio- or chemotherapy, is hindered by the chemoresistance or secondary effect. Due to the complex and dynamic interactions in tumor microenvironment, there is constant need in designing new anti-cancer strategies that simultaneously target directly cancer cells development and indirectly the pro-tumor processes mediated by the crosstalk of cells in tumor millieu. The aim of our study was to investigate the cytotoxic actions of curcumin combined with 5-fluorouracil (5-FU) against C26 murine colon cancer cells co-cultured with macrophages and to elucidate the mechanisms underlying their effects. Curcumin, an anti-oxidant and anti-inflammatory agent, was administered prior to 5-FU treatment, to sensitize the cancer cells to this cytotoxic drug. The impact of these two agents on cell proliferation capacity, key oxidative stress, inflammatory, and angiogenic markers was evaluated, under normoxic or hypoxic culture conditions. Our preliminary data revealed that, after 48 h of treatment, curcumin (17 μM) combined with 5-FU (2 μM) inhibits C26 cancer cell proliferation (>80% compared to control) in both monoculture and co-culture system, in normoxia, as shown by BrdU incorporation assay. This inhibitory effect was maintained in hypoxic conditions, but only with the use of higher concentrations of curcumin (100 μM), in both monoculture or co-culture systems. No further improvement of these cytotoxic effects were seen when combined *vs.* individual administration of these agents was applied. Further experiments should address the role of macrophages in orchestrating these anti-tumor actions of curcumin and 5-FU. Our results may offer detailed information on how the tumor-associated processes emerging from the malignant crosstalk of macrophages and colon cancer cells can be modulated for future anti-cancer therapeutic approaches.

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[P 2.17] TOCOPHEROLS AND CAROTENOIDS IN BERRIES IN RELATION WITH ANTIOXIDANT PROPERTIES

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Wild and cultivated berries such as *Rosa canina* L. (rosehip), *Lycium barbarum* L. (goji) or *Hippophae rhamnoides* L. (sea buckthorn), are largely used as food or supplements due to their well documented health properties. A diet rich in fruits is associated with a lower risk of degenerative and inflammatory diseases and lower prevalence of cancer. Tocopherols and carotenoids are bioactive compounds with a significant contribution to the antioxidant capacity of fruits.

The aim of this study was to determine the composition and the level of tocopherols, tocotrienols and carotenoids in above mentioned berries and to determine their antioxidant capacity. Tocopherols were determined by HPLC on normal phase column and fluorescence detection. Carotenoids were analyzed in unsaponified and saponified extracts by HPLC on C30 column and photodiode array detector. Total phenolic compounds were quantified by a spectrophotometric assay and the antioxidant capacity was determined by TEAC and DPPH methods.

Three tocopherols (α, δ and γ) and the corresponding tocotrienols were identified in all samples. The total amount of tocopherols and tocotrienols were 29.7, 48.54 and 53.15 mg/kg (F.W) in goji, rosehip and respectively sea buckthorn. The major compound was α-tocopherol, which represented 73-82 % of total tocols, followed by α-tocotrienol and γ-tocopherol. Total carotenoid content was 25.31, 16.47 and 15.1 mg/100 g (F.W.) in goji, rosehip and sea buckthorn. The major compounds were zeaxanthin esters (goji and sea buckthorn), lycopene (rosehip) and β-carotene (rosehip and sea buckthorn). The antioxidant capacity of the extracts ranged between 2.4-3.5 micromoles TE/g (DPPH) and 1.87-9.61 micromoles TE/g (TEAC). Better correlations were found between the antioxidant capacity and the content in tocopherols and polyphenols than for carotenoid content.

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[P 2.18] **FORMULATION AND CHARACTERIZATION OF NEW PHYTOCOSMETICS WITH DERMOPROTECTIVE EFFECTS**

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Herbal extracts are wellknown as prophylactic as well therapeutic and health promoting agents. Cosmetic products including bioactive phytochemicals may provide improved physical barrier and tissue protection against external environment[1].

The purpose of this study was to produce new cosmetic formulations which contain, beside olive oil, bioactive sea buckthorn or apricot seed oils, rich sources of antioxidants, such as carotenoids, flavonoids and vitamins with dermoprotective effects. Five cosmetic formulations with hydrating effects were obtained as oil-in-water (o/w) emulsions, containing 34% oil phase, 64% hydrophilic phase and also a bioactive phase of vitamins and ingredients, sensitive to high temperatures.

These formulas were characterized by their sensorial features, and evaluated for their antioxidant capacity (using DPPH and TEAC methods). FTIR spectrometry (400 - 4000 cm^{-1}), a non-destructive method very useful to be used for cosmetics quality assessment[2,3] was applied also to the ingredients used in these products and absorption bands were identified, corresponding to types of bonds and functional groups. The antioxidant capacity of these formulas ranged between 408 and 504 μm Trolox equivalents/g sample (by DPPH method) and between 0.05 - 0.7 μm Trolox equivalents/g sample (by TEAC method).

The unsaturation, peroxidation and carbonyl indices of the five formulas studied were calculated from the data obtained by FTIR spectrometry. The specific frequencies of the functional groups and fingerprint areas were identified (zone IV), e.g. the specific ones for antioxidant carotenoids, unsaturated fatty acids and tocopherols. The zone III identified the presence of saturated fats or esters (1100-1800cm^{-1}), while zone II was specific to carbonyl derivatives (2800cm^{-1}) .

The use of FTIR has allowed the fingerprinting of the active principles, to be compared among the five different creams, identifying similar shapes and specific recognition of ingredients inside each cosmetic formulation.

All these data offer good opportunities for creating innovative formulas of cosmetics with improved dermoprotective action.

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[P 2.19] AMINO ACIDS AS SPECIFIC URINARY BIOMARKERS FOR BREAST CANCER METABOLOMICS

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Metabolomics is considered the "apogee" of omics' technologies, fingerprinting and identifying changes of all biofluid or tissue small metabolites, as a mirror of specific diseases or treatments monitoring[1]. Metabolomic analysis includes two experimental approaches, a non-targeted and/or targeted analysis. Targeted metabolomics identifies and quantifies a defined number of metabolites, usually around 20 small metabolites from one or more specific metabolic pathways[2]. Amino acids from blood or urine are key metabolites used in targeted analysis and may reflect the health conditions or a disease development. The most adequate technique for amino acids analysis is HPLC- QTOF-ESI(+)-MS[3].

The aim of this study was to determine and quantify urine free amino acid LC-MS profiles from breast cancer patients in order to investigate their potential as predictive biomarkers, after an adequate multivariate analysis of experimental data.

Thirty urine samples were collected from patients histologically diagnosed with breast cancer in different stages, comparatively with five control patients. Amino acids were extracted and purified using a solid phase extraction step, followed by pre-column derivatization and liquid/liquid extraction according to EZ-Faast kit (Phenomenex), the derivatized samples were analyzed by LC-QTOF-(ESI+)-MS. The control of the instrument and data processing was done using TofControl 3.2 and Data Analysis 4.2 (BrukerDaltonics). After processing the data, the chemometric analysis (PCA and Cluster Analysis) were conducted by Unscrambler10.1 software.

A number of sixteen free amino acids were separated and quantified, their chemometric analysis showed differences between breast cancer patients and healthy controls, the group of essential amino acids being more representative as biomarkers for a good discrimination among patients groups. Cluster analysis revealed three patient groups characterized by different amino acid concentrations, the control group having intermediate values of amino acids. The Principal Component Analysis revealed that PC1 *vs* PC2 score described 90% of variation, so a high prediction value. Good discriminations were noticed between pathologic and control samples, with inferior values of phenylalanin, serine, valine, glutamic acid, glumatine and hystidine. The aminoacids which contribute to the sample discriminations are tyrosine, serine, isoleucine and lysine.

Correlations between the urine and blood aminoacid profiles as well additional processing data for larger patients cohorts are under evaluation.

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[P 2.20] CYTOTOXIC EFFECTS OF CURCUMIN AND 5-FU ON MURINE COLON CANCER CELLS CO-CULTURED WITH MACROPHAGES

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Colon cancer is a severe type of disease, in which surgical therapy complemented by radio- or chemotherapy, is hindered by the chemoresistance or secondary effect. Due to the complex and dynamic interactions in tumor microenvironment, there is constant need in designing new anti-cancer strategies that simultaneously target directly cancer cells development and indirectly the pro-tumor processes mediated by the crosstalk of cells in tumor millieu. The aim of our study was to investigate the cytotoxic actions of curcumin combined with 5-fluorouracil (5-FU) against C26 murine colon cancer cells co-cultured with macrophages and to elucidate the mechanisms underlying their effects. Curcumin, an anti-oxidant and anti-inflammatory agent, was administered prior to 5-FU treatment, to sensitize the cancer cells to this cytotoxic drug. The impact of these two agents on cell proliferation capacity, key oxidative stress, inflammatory, and angiogenic markers was evaluated, under normoxic or hypoxic culture conditions. Our preliminary data revealed that, after 48 h of treatment, curcumin (17 μM) combined with 5-FU (2 μM) inhibits C26 cancer cell proliferation (>80% compared to control) in both monoculture and co-culture system, in normoxia, as shown by BrdU incorporation assay. This inhibitory effect was maintained in hypoxic conditions, but only with the use of higher concentrations of curcumin (100 μM), in both monoculture or co-culture systems. No further improvement of these cytotoxic effects were seen when combined vs. individual administration of these agents was applied. Further experiments should address the role of macrophages in orchestrating these anti-tumor actions of curcumin and 5-FU. Our results may offer detailed information on how the tumor-associated processes emerging from the malignant crosstalk of macrophages and colon cancer cells can be modulated for future anti-cancer therapeutic approaches.

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[P 2.21] RABBIT PRIMARY CHONDROCYTE-BASED MODEL FOR TESTING THE THERAPEUTIC POTENTIAL OF NATURAL COMPOUNDS FOR OSTEOARTHRITIS

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Osteoarthritis is a degenerative disease that affects the joints of aging people. One of the goals of our company is to find natural compounds for the treatment of osteoarthritis. The screening of new compounds requires reliable methods for determining the potential therapeutic value of the tested substances.

Ideally the in vitro model used in the preliminary research should be as close as possible to the in vivo conditions. We chose rabbit primary chondrocytes as a model for our experiments considering the accessibility of this source and the moderate level of difficulty to obtain primary cultures.

The chondrocytes are separated from articular cartilage of the long bones of the rabbit and the effects of osteoarthritis are mimicked in culture by treatment with IL-1β. The treatment with IL-1β leads to increased levels of the pro-inflammatory molecules and of the enzymes implicated in the degradation of the cartilage matrix and to decreased expression of the matrix molecules. The compounds are tested in this setup and the ones that diminish or reverse the effect of the IL-1β treatment are considered hits and are further evaluated.

Our results show that this model is very reliable. Using this model we found a number of compounds that show therapeutic potential.

[P 2.22] ANTIOXIDANT ACTIVITY OF RIBOFLAVIN IN POLYETHYLENE GLYCOL - BOVINE SERUM ALBUMIN SYSTEMS

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Riboflavin (RF), a hydrosoluble and biocompatible vitamin, Vitamin B_2, has a special role in redox processes occuring in humans. The work is an extension of the previous papers[1-3] and aims to simulate *in vitro,* the effects caused by oxidation of RF in Polyethylene Glycol (PEG)(Tween20, L64, Myrj52)-Bovine Serum Albumin (BSA) systems, using the chemiluminescent system luminol-hydrogen peroxide, in Tris-HCl buffer, pH 8.5. The tested concentrations were 2÷20 μM for RF and 0.06÷0.6 % for PEGs. It was found that 0.12 % PEGs enhance the antioxidant activity of RF to higher and lower values, according to their molecular structure, as follow: Tween20 > Myrj52 > L64. RF has a prooxidant effect in the presence of BSA, while in the PEG-BSA systems, its antioxidant activity increases up to ~20 %; A cross-linking of PEGs to RF in the presence of BSA is considered. Additionaly, studies by fluorescence spectroscopy on RF embedded in Tween20-BSA and Myrj52-BSA systems showed a slight Tyrosine fluorescence contribution. The results are discussed with relevance to the oxidative stress and drug delivery processes.

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NANOSTRUCTURES AND BIOMEDICAL APPLICATIONS

[P 3.1] **WARFARIN REDUCED GOLD NANOPARTICLES: SYNTHESIS, CELLULAR UPTAKE AND CYTOTOXICITY**

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The application of gold nanoparticles in the biomedical field is receiving increased attention [1]. Various preparation techniques have been reported so far. Even if there is a wide range of materials used for producing gold nanoparticles there is a continuous need for the development of new protocols. Moreover, the applicability of such nanoparticles needs further investigation. Their biocompatibility, cellular uptake and biodistribution are important aspects that require careful assessment. Here we report the synthesis of gold nanoparticles using the anticoagulant sodium warfarin as reducing agent for the tetrachloroauric acid ($HAuCl_4$). The nanoparticles were characterized by UV-Vis, FTIR and Raman spectroscopy, and transmission electron microscopy. Their cytotoxicity and cellular uptake were evaluated on two different cell lines, the human fetal lung fibroblast (HFL-1) and human retinal epithelial (D407). UV-Vis spectroscopy confirmed the formation of gold nanoparticles by the presence of surface plasmon resonance bands at 533 nm. The nanoparticles showed polygonal shape and 50-70 nm diameter. FTIR showed surface modification of the nanoparticles with sodium warfarin. Transmission electron microscopy proved that the gold nanoparticles were able to cross the cellular membrane of the HFL-1 and D407 cells. The nanoparticles were localized in the cell cytoplasm and they entered the cells via endocytosis. Cellular viability assays performed using the MTT assay showed that the nanoparticles were non-cytotoxic after 24 and 48 h of cell incubation in the presence of different nanoparticle concentrations.

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[P 3.2] **CELULAR UPTAKE AND ACTIVITY OF CERIUM OXIDE NANOPARTICLES IN VITRO**

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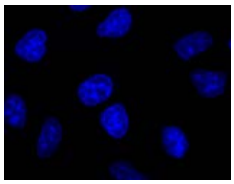
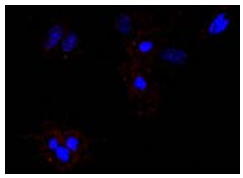
Nanotechnology is becoming a very important field in biomedical and clinical research. The use of nanoparticles offers new possibilities to prevent, diagnose and treat many diseases, including cancer. Several nanoparticles such as silver, gold, zinc or cerium oxide have been approved and used in clinical treatments. [1]. Nanoparticles having size range of 2–100 nm can interact with biological systems at the molecular level and the release of active compounds can be controlled [2]. Rare earth metallic cerium oxide nanoparticles (*CeO2-NP*) are known for their ability to react with oxygen free radicals. Being involved in redox reaction *CeO2-NP* can act specifically towards to cancer and normal cells and also they can be used as drug delivery or alternative chemotherapy agents.

The aim of this work was to investigate the cellular uptake and antiproliferative potential of *CeO2-NP* having 5- 20 nm on A549 (human lung cancer cell line).



Control



Treatment

Figure 1. Cellular uptake of CeO2 NPs on A549 cell line by confocal microscopy, control cells without nanoparticles cells treated with Rhodamine 123 conjugated nanoparticles. Cells nuclei are stained with Draq 5

Cytotoxicity was evaluated using the 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide reagent (MTT assay) and cellular uptake was evaluated and confirmed using a fluorescent dye – Rhodamine123, which is a member of the rhodamine family of fluorone dyes. Monitoring of *rhodamine uptake* was carried out by fluorescence microscopy.

The obtained results demonstrate that nanosized CeO2-NP are cytotoxic against HeLa cell line as analyzed by MTT cell proliferation assay. Cellular uptake was confirmed by confocal microscopy (Fig. 1). The obtained results showed a dose dependent and exposure time decrease of viability.

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[P 3.3] **LASER BASED METHODS FOR FUNCTIONAL SURFACE ENGINEERING**

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Micro-/nanoengineering of surfaces for manipulating the cellular behavior at the cell–biomaterial interface *in vitro* are of interest in various domains, from cell basic studies to tissue engineering. Among various techniques used for surface engineering, laser based approaches are new and interesting strategies for designing controlled bio-interfaces.

In this work, surfaces modified with polymers or biological compounds with multiple functionality (from protein repelling characteristics to antitumoral activities and *smart* coatings) were obtained by Matrix Assisted Pulsed Laser Evaporation-MAPLE, Laser Induced Forward Transfer-LIFT and direct laser texturing. A focus is placed on general descriptions of laser methods parameters as guiding tool for how to best obtain desired micro or nano-patterns or coatings. Finally, the main issues and challenges in addressing key limitations associated with current laser micro-patterning technology are discussed and various examples demonstrating that direct cell–material contacts can be used to study complex and dynamic relationships between cells and their environment.

Acknowledgments. The research leading to these results has received funding from the Romanian Ministry of National Education, CNCS – UEFISCDI, under the project PN-II-PT-PCCA 239/2014

[P 3.4] **THE SIGNIFICANCE OF DETERMINING ADENOSINE DEAMINASE IN PLEURAL FLUID AND BLOOD**

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Introduction. Adenosine deaminase (also known as adenosine aminohydrolase, or *ADA*) is an enzyme that catalyzes the hydrolysis of purine adenosine to inosine and ammonia. An increased activity of ADA in pleural fluid and serum is useful in evaluating pleural TB infection, along with other investigations. The study covered biochemical and cytological investigation of pleural fluid and blood from patients in the TB ward.

Material and methods. There were surveyed 212 patients aged between 2 months and 92 years of which 120 male and 92 female. In most patients, i.e. 68.98% (142 patients) ADA was determined in pleural fluid, in 33.01% (70 patients of which 41 children) ADA was determined in blood and in 1.88% (4 patients) ADA was determined in both pleural fluid and blood. Cytology was performed on pleural fluid Giemsa stained smears ***Results.*** ADA values in pleural fluid were above the reference value (>33U/L) in the case of 52.81% of all patients (75 patients); ADA values in pleural fluid were above the reference value (>18 U/L) in the case of all patients investigated (70). *Cytology* in patients with ADA above the reference value showed various types of inflammation modifications, as follows: slightly reactive in 4 patients, reactive in 70 patients and intensely reactive in 1 patient. In 29.71% of total patients (63 patients) lymphocytes were >90% and in 6 patients cytology showed empyema.

Conclusions. Increased value of ADA in pleural fluid and blood (in patients with no pleural effusion) and lymphocyte predominance in the clinical context guides rapidly the diagnosis to a specific aetiology.

[P 3.5] *IN VITRO* STUDY OF PON1 ACTIVITIES RESPONSE TO ATYPICAL ANTIPSYCHOTICS

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Serum paraoxonase (hPON1; EC 3.1.8.1) is an HDL-associated enzyme considered to play a major role in the control of oxidative stress and inflammatory response in circulation and endothelial vascular wall.

In this study we have investigated the effects of risperidone, an atypical antipsychotic drug used to treat psychotic conditions such as autism and schizophrenia, on paraoxonase and arylesterase activities of serum hPON1.

Lineweaver-Burk plots showed that risperidone exerts a non-competitive inhibition of arylesterase activity, but a competitive inhibition of paraoxonase activity. These results could partially explain decreased hPON1 activities observed in our previous studies on autistic children.

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[P 3.6] *IN VITRO* BIO-FUNCTIONAL PERFORMANCE OF LACTOFERRIN EMBEDDED COMPOSITE COATINGS OBTAINED BY MATRIX ASSISTED PULSED LASER EVAPORATION

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One approach for improving the efficacy of metal scaffold in bone integration and tissue repair is engineering the surface by embedding proteins known to have a physiological role in bone growth. Lf is a multifunctional protein known to be involved in the immune response (cytokine release) and the regulation of bone regeneration.

In this work, the feasibility of using lactoferrin (Lf) embedded in the Poly (ethylene glycol)-block-poly (caprolactone) methyl ether –hydroxyapatite (HA) composite matrix as potential anti-inflammatory coating was explored. The films were deposited using Matrix Assisted Pulsed Laser Evaporation (MAPLE). Fourier Transform Infrared Spectroscopy (FTIR), contact angle measurements and Atomic Force Microscopy (AFM) were used for establishing the chemical and morphological characteristics of the films. The data demonstrated that the functional groups in the MAPLE-deposited films remain intact while the homogeneity and the roughness of the coatings are related to target composition and laser parameters.

Using a combination of analysis methods including non-radioactive cell proliferation assay and enzyme-linked immunosorbent assay (ELISA) on an *in vitro* model of inflammation we characterized inflammatory response of biomaterials. No cytotoxic effect onto THP-1 cells treated or untreated with LPS for 18 hours was revealed. The number of cell attached on coated surfaces with lactoferrin and hydroxyapatite was higher than their counterparts grown on films embedded with HA or Lf alone, suggesting a better modulation of the cell attachment. However, LPS addition led to a decrease in the total number of cells irrespective of surface covering. Proinflammatory cytokine TNF-α released from macrophages was observed only in the case of endotoxin treatment, the lowest amount of cytokine being detected in the case of Lf-HA functionalized materials.

Our results revealed a promising early immunomodulatory response of the bioactive Lf-HA functionalized materials and a better performance in sustaining future osseous implant integration.

Acknowledgments. The research leading to these results has received funding from the Romanian Ministry of National Education, CNCS – UEFISCDI, under the project PN-II-PT-PCCA 239/2014

[P 3.7] **CERIUM OXIDE NANOPARTICLES AS ANTIMICROBIAL AGENTS AGAINST PATHOGENIC BACTERIA *IN VITRO***

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Antibiotics or antimicrobials have been widely used in food industry for pathogens eradication and foodborne prevention. Nevertheless, lately a great concern is arising related to multiple drug resistant microorganisms and to the safety of the preservatives utilized in foods. A great effort of worldwide scientists is directed towards finding new alternatives for combating pathogens.

The utilisation of cerium oxide nanoparticles (CeO_2NPs) as antibacterial agent was proposed and tested on five pathogens, namely: *Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphyloccocus aureus* and *Bacilus cereus*.

In this research the synthesis of CeO_2NPs was conducted using the precipitation method. The nanoparticles were characterised by spectroscopic and microscopic methods and their antioxidant and antibacterial activity was tested on two gram negative and three gram positive pathogens respectively. For the antimicrobial activity the well diffusion method and UV-VIS spectrometry (optical density) were used.

Small size nanoparticles were obtained by the precipitation method. The antibacterial tests evidence that the inhibitory effect of CeO_2NPs is present at lower concentration than the control (standard drug). Confocal microscopy was utilized to confirm the obtained results. The time kill assay shows an inhibitory effect in a time dependent manner, indicating that CeO_2NPs interaction with the tested pathogens results in bacterial cell damage.

The experimental results prove that the CeO_2NPs exhibit excellent antibacterial activity against the five tested bacterial species: *E. coli, S. typhimurium, L. monocytogenes, S. aureus* and *B. cereus.*

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[P 3.8] **INTERACTION OF HUMAN PRIMARY CELLS WITH BIOMATERIALS DESIGNED FOR BIOMEDICAL APPLICATIONS**

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The main focus of my research in the last ten years was the understanding of processes involved at cell – biomaterials interfaces during *in vitro* interactions in order to guide the design and biofabrication of nano/micro-structures for biomedical applications. These investigations were initiated and continue even presently in collaboration with chemists and physicists interested in the development of new materials for tissue engineering. Global increase in life expectancy has generated the need for long lasting bone prosthesis. At first, I was involved in isolation of human bone marrow-mesenchymal stem cells (MSCs) and characterization of attachment, proliferation and differentiation to osteoblasts onto hydroxyapatite (HA)-based thin films (1, 2). We have determined that bone cells sense differences in materials chemistry by activating the MAPK pathway, upon attachment to gradients of biomolecules deposited by an innovative combinatorial approach using laser technology (3). Using image analysis, we have quantified the effects of MSCs guidance on microtopographies with controlled geometries obtained by femtosecond laser irradiation of zirconia for use in cochlear implantology. Our study showed that cells and nuclei were elongating function of the distance of the designed peaks and troughs. Furthermore, we performed co-cultures of primary skin cells on a 3D ormosil scaffold that we proposed to be used for dermal graft construction (4). More recently, we participated in characterization of umbilical cord-derived MSCs potentiality for use in clinical therapy (5). Currently, the interest of our work is to increase the efficiency of osteogenic differentiation using growth factors embedded in a 3D hierarchical polymeric scaffold for long bones implants. Hence, we present here a retrospective view of our research in the field of nanotechnology for medicine.

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[P 3.9] *IN VITRO* EVALUATION OF BIODEGRADABLE POLYMERIC NANOPARTICLES AS DRUG DELIVERY DEVICES

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Polymeric nanoparticles (PNPs) have been proven to enhance the cellular uptake of entrapped bioactive compounds, to improve the drug efficacy, and to possess better stability, relative to alternative delivery systems. Poly(D,L-lactide-co-glycolide) (PLGA) is a synthetic polymer used in biological applications, approved by the Food and Drug Administration (FDA) and generally recognized as safe (GRAS) for pharmaceutical applications. Chitosan-coated PLGA particles (Chi–PLGA) were developed to utilize the mucoadhesive property of chitosan and PLGA's ability to efficiently entrap hydrophobic and hydrophilic drugs. Biocompatibility and intracellular uptake of PNPs (120–220 nm) carrying negative (PLGA) and positive charges (Chi-PLGA) were studied in different cell types (mouse embryonic fibroblasts - K41, mouse melanona cell - B16-F10, human hepatoma cell-HepaRG and epithelial cells from bovine kidney-MDBK) in order to develope a novel drug delivery systems usefull in metabolic disorders or cancer therapy. PNPs interaction with mammalian cells was assessed by cytotoxicity measurement, fluorescence microscopy investigation, and flow cytometry quantitation. PLGA based NPs showed extremely low cytotoxicity only when delivered in high concentrations (over 2500μg/mL), while an increase of the cell proliferation was observed for PLGA-Chi NPs. PLGA-Chi is more potent than PLGA to pass membrane barriers but demonstrated increased tendency to aggregate, which could interferes with their potential function as nutrient/drug carriers. Further studies are needed to determine ideal conditions required for PNPs to enter the cells and their intracellular localization. Our data are significant for the potential applications of PLGA-based NPs as nutrient delivery system or anti-tumor drug delivery vehicle.

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[P 3.10] CHEMOMETRIC ANALYSIS FOR THE EVALUATION OF GLUCOSINOLATES PATTERNS IN *BRASSICA* VEGETABLES DURING FERMENTATION

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Glucosinolates (GLS) are plant secondary metabolites, which are present in all Cruciferous vegetables, mainly in *Brassica* vegetables which includes a large variety of horticultural crops, appreciated by their nutritional properties and multiple health benefits. During the food processing of *Brassica* vegetables, the total GLS content decreases, three mechanisms being involved: (i) enzymatic breakdown involving myrosinase, a thioglucoside glycohydrolase (EC 3.2.3.1), (ii) leaking of GLS into the cooking water and (iii) thermal degradation.

The lactic fermentation of vegetables is one of the oldest preservation technologies, which yields palatable food, which either retains a part of the original nutrients or new ones are formed, in a probiotic environment (lactic microorganisms). The fermented vegetables can be preserved for long periods of time, without refrigeration, especially when herbs, spices and other ingredients are added to improve the stability, aroma and flavor. The aim of our study was to investigate the impact of homemade lactic fermentation (in salt and acetic acid or with salted water) on the level of individual GLS, from four different *Brassica* vegetables (broccoli, cauliflower, white and red cabbage), using advanced chromatographic separation by HPLC-PDA (High Performance Liquid Chromatography coupled with Photo Diode Array) assisted by chemometric analysis. The highest total GLS content was noticed in broccoli, followed by red and white cabbage, and cauliflower (4.93, 3.35, 3.15 and 2.07 μmol/g dw, respectively). After lactic fermentation, the most affected were indole glucosinolates, excepting the white cabbage, where total aliphatic glucosinolates decreased strongly. Among individual glucosinolates, 4-hydroxy-glucobrassicin increased by 27% and 39% during fermentation in salt/acetic acid and with salted water, respectively.

This study has shown that individual glucosinolates content decreased substantially after lactic fermentation and as demonstrated by Multivariate Statistical Analysis, glucosinolates profiles can be considered as significant discrimination factors to evaluate the different fermentation processes of *Brassica* vegetables.

[P 3.11] HALOARCHAEAL PRODUCTION OF SILVER NANOPARTICLES WITH APPLICATIONS AS ANTIMICROBIAL AGENTS

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Introduction. There is an acute demand for the discovery of novel categories of antimicrobial agents that might aid in the treatment of infectious diseases triggered by antibiotic-resistant bacteria. Silver nanoparticles (SiNPs) are known for their efficient antimicrobial activity, and their biosynthesis could be a practical, economical and non-toxic way of production. Recently, members of the halophilic archaea belonging to *Halobacteriaceae* family were documented for their capacity to produce SiNPs under high salinity [1], [2] thus expanding our knowledge on the biogenesis of SiNPs in prokaryotes.

In this study, we explored the capacity of two strains of halophilic Archaea (*Haloferax alexandrinus* and *Halomicrobium mukohataei*) to tolerate high concentrations of silver (as $AgNO_3$), by producing SiNPs.

Materials and Methods. Strains were cultured on media with 17-25% NaCl and 0.5 mM $AgNO_3$. Their ability to grow at high Ag concentration was monitored by change in optical density whereas the capacity to produce SiNPs was illustrated by EDX/SEM analyses.

Results and Discussions. After being exposed to the above-mentioned conditions for 5 days, the strains produced a dark precipitate. The EDX and SEM analysis indicated the presence of silver particles in the media. A possible mechanism that would explain the capacity to tolerate silver in tested microorganisms is the reduction of $AgNO_3$, involving *nitrate reductase*, an enzyme presumed to be a component of the respiratory chain.

Conclusions. SiNPs can act as efficient antibacterial and antifungal agents at low concentrations that do not affect mammalian cells. The growth of archaeal microorganisms under high salt concentration and in the presence of $AgNO_3$ might prove a rapid and efficient method to obtain silver particles under conditions that could prevent the contamination of microbial cultures.

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[P 3.12] **ANALYSIS OF MELANOMA CELL POPULATIONS BY TISSUE FAXS**

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Metastatic melanoma is the deadliest skin cancer. Within heterogenic tumor cell populations, cell subsets with enhanced tumorigenicity are suspected of high aggressivity and drug-resistance. The distribution of tumor antigens in relation with different markers associated with malignant progression within tumor cell populations can give more insights into specific mechanisms which initiate and perpetuate this type of cancer. Tissue FAXS image cytometry system allows the quantitative analysis of immunofluorescent labeled antigens in cytological fixed specimens.

Our goal was to investigate the expression of DOPAchrome tautomerase (DCT), a melanoma antigen and mediator of an anti-apoptotic, therapy-resistant tumor cell pathway in a human melanoma cell line with increased proliferative potential in relation with a scaffold protein, major regulator of signaling platforms in melanoma cell subsets.

To this view, cells were cultured for different time periods on cover glass supports, fixed and double stained using standard immunolabeling. We found that DCT expression is highly dependent on culture stage and environmental conditions and is not uniformly distributed within the whole culture cells. Tumor subsets with low-, medium- or high- DCT expression levels were identified. We also established that DCT expression is inversely correlated with a specific protein marker of cholesterol rich membrane subdomains.

This experimental set up will allow further separation, culture and analysis of DCT-high clones.

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