Serbian Biochemical Society
Ninth Conference

"Diversity in Biochemistry"

Proceedings

Faculty of Chemistry – Kolarac Endowment
Belgrade 2019

Serbian Biochemical Society

Proceedings

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Serbian Biochemical Society
Ninth Conference

with international participation

University of Belgrade – Kolarac Endowment
14-16.11.2019. Belgrade, Serbia

"Diversity in Biochemistry"

PROGRAMME

Thursday	**November 14th, 2019**	**Kolarac Endowment**

10:00-18:00	Registration	
11:30-12:00	Opening ceremony	
12:00-14:10	Session 1	
12:00-12:40	Snežana Đorđević (Plenary Lecture (PL) 1)	
	University College London, United Kingdom	(35+5)
	Proteins that sense cellular environment – examples and implications	
12:40-13:10	Milica Popović (Invited Lecture (IL) 1)	
	Faculty of Chemistry, University of Belgrade, Serbia	(25+5)
	Routine and novel methods for isolation of extracellular vesicles	
13:10-13:40	JelenaVekić (IL2)	
	Faculty of Pharmacy, University of Belgrade, Serbia	(25+5)
	The role of proprotein convertase subtilisin/kexin type 9 in atherosclerosis	
13:40-14:10	Danijela Cvetković (IL3)	
	Faculty of Science, University of Kragujevac, Serbia	(25+5)
	The role of molecular markers of angiogenesis in the prediction disease in patients with breast cancer	
14:10-16:10	Lunch break / **Poster sessions 1 and 2**	
16:10-18:00	Session 2	
16:10-16:40	Jelena Dragišić Maksimović (IL4)	
	Institute for Multidisciplinary Research, University of Belgrade, Serbia	(25+5)
	Longitudinal distribution of apoplasticantioxidative components in maize root	

16:40-17:05	Dr. Jakub Nowak (Sponsored Lecture (SL) 1) NanoTemper Technologies, Poland (20+5) **When proteins matter – beyond interactions and protein stability**
17:05-17:20	Dragan Malenović (SL2) Analysis doo, Belgrade, Serbia (10+5) **Analysis d.o.o – Commercial presentation**
17:20-18:00	**5 Speed talks from selected abstracts** (5+3)
18:00-	Welcome party

Friday	**November 15th, 2019**	**Kolarac Endowment**

10:00-10:40	FEBS National Lecture
10:00-10:40	Dejana Mokranjac (PL2) Ludwig-Maximilians-University, Germany (35+5) **A journey along the TIM23 complex, the major protein translocase of the mitochondrial inner membrane**
10:40-11:20	Coffee break
11:20-13:20	FEBS3+ Meeting Session
11:20-11:50	Nataša Poklar Ulrih (IL5) Biotechnical Faculty, University of Ljubljana, Slovenia (25+5) **Molecular adaptation to high temperatures: Pernisine from archaeon *Aeropyrum pernix* K1**
11:50-12:20	Lajos Haracska (IL6) Biological Research Centre, HAS, Szeged, Hungary (25+5) **Engines of mutagenesis and carcinogenegesis; replication of damaged DNA**

12:20-12:50	Zrinka Kovarik (IL7)
	Institute for Medical Research and Occupational Health, Zagreb, Croatia (25+5)
	Detoxification of nerve agents by oxime-assisted reactivation of cholinesterases
12:50-13:20	Ivana Momčilović (IL8)
	Institute for Biological Research "Siniša Stanković, University of Belgrade, Serbia (25+5)
	Elongation factors Tu and 1A – multifunctional proteins involved in plant heat tolerance
13:20-15:20	Lunch break / **Poster sessions 3 and 4**
15:20-16:30	FEBS Umbrella Session
15:20-15:30	Jerka Dumić
	FEBS WGI (5+5)
	FEBS Activities and Fellowships
15:30-16:00	Ferhan Sagin (IL9)
	FEBS Education Committee (25+5)
	How to use educational technology to make education better - Not just different or entertaining!
16:00-16:30	Irene Diaz Moreno (IL10)
	FEBS WGCareers of Young Scientists (25+5)
	How FEBS supports young scientists´ career
16:30-18:00	Session 6
16:30-16:45	Igor Pongrac (SL3)
	Merck Life Science | Research Solutions (10+5)
	Rethink Western blotting with Merck

16:45-17:00	Dušan Dunjić (SL4) Biologist group doo, Belgrade, Serbia (10+5) **Endotoxin detection-LAL test, fast, easy to use and competitive**
17:00-17:15	Nebojša Dovezenski (SL5) LKB Vertriebs doo, Belgrade, Serbia (10+5) **Chromatography system AKTA go, latest addition to AKTA range systems, and recently marketed Amersham™ ImageQuant™ 800 camera based bioimager including infra red (IR) short and IR long capabilities: what is new here and how this can help you in research?**
17:20-18:00	**5 Speed talks from selected abstracts** (5+3)
19:30-	Conference dinner

Saturday November 16th, 2019 Faculty of Chemistry

10:00-11:10	Session 7
10:00-10:30	Marija Janjić (IL11) Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia (25+5) **Gonadotropin-releasing hormone regulated transcription of gonadotropin subunit genes**
10:30-11:10	**5 Speed talks from selected abstracts** (5+3)
11:10–11:30	Coffee break
11:30-12:00	Session 8
12:00-12:30	Romana Masnikosa (IL12) Vinča Institute of Nuclear Sciences, University of Belgrade, Serbia (25+5) **Metallomics and mass spectrometry for drug development: employing ICP-OES and MALDI TOF MS for assessing protein-drug interactions**

12:30-13:00	Nataša Simin (IL13)
	Faculty of Sciences, University of Novi Sad, Serbia (25+5)
	An overview of biological activities of less known wild onions (genus *Allium* sect. *Codonoprasum*)
13:00-13:30	Aleksandar Veselinović (IL14)
	Faculty of Medicine, University of Niš, Serbia (25+5)
	The application of *in silico* methods in biochemistry
13:30-14:00	Closing ceremony; The best poster award

15:00-18:30	**Workshop on microscale termophoresis (hands-on approach) organized by Nanotemper at University of Belgrade Faculty of Chemistry**
15:00-16:00	Registration
16:00-16:30	Dr. Jakub Nowak
	When interactions matter
16:30-17:00	Małgorzata Poczopko
	When data quality matters. MST assay development and data analysis.
17:00-18:30	Samples discussion and preliminary preparations

Sunday	**November 17th, 2019**	**Faculty of Chemistry**

09:00-13:00	Experimental work
13:00-14:00	Lunch break
14:00-17:00	Experimental work
17:00	MST Workshop End

Poster Sessions

Poster Session 1 - General and Analytical Biochemistry

Sofija Bekić

Optimization of ligand binding assay in yeast for identification of phytoestrogens with affinity for estrogen receptor β

Maja Ćupurdija

Comparative study of different DNA isolation methods from plants and fungus

Lidija Filipović

Development immunoaffinity chromatography for purification extracellular vesicles

Oginni Gbenga Folorunsho

Quantitative detection of *Microcystis aeruginosa* in fresh water using single domain antibodies (VHHs)

Marianna Holczer

Fine-tuning of AMPK-ULK1-mTOR regulatory triangle is crucial for periodic activation of autophagy

Zorana Lopandić

BanLec-GFP binding to influenca virus glycans

Jovana Lukičić

Effects of vanadate on glutathione metabolism in mycelium of fungus *Phycomyces blakesleeanus*

Maja Marinović

Identification of novel bile acid derivatives as 3α-HSD III inhibitors

Margita Márton

The dynamical characteristic of PERK targets choosing between life and death upon endoplasmic reticulum stress

Jelica Milošević

Kinetics of amyloid fibril formation in the presence of metal ions and low-molecular-weight compounds

Andrijana Nešić

Employment of mouse-derived intestinal 2D organoids for evaluation of major kiwi-fruit allergen Act d1 effect on intestinal epithelial cells

Aleksandra Nikezić

Comparative analysis of DNA extraction methods from human buccal swabs and fish tissue samples

Sandra Oloketuyi

Functionalized nanobodies: A bio-recognition molecule for the detection of the toxic microalgae Alexandrium minutum by means of an electrochemical immunosensor

Ana Penezić

Serum transferrin glycopattern in patients with an end-stage renal disease: a lectin-based protein microarray

Anđela Platiša

Isolation of anti-extra-cellular vesicle single-domain antibodies

Zsuzsánna Réthi-Nagy

Developing a novel protein tagging, immunodetection and purification system

Anna Somogyi

Fatty acid profiling in cultured cells by using gas chromatography – flame ionization detection

Orsolya Szatmári

Phase separation, an important level of gene expression regulation

Ferhan Sağın

Determination of serum hypoxia-inducible factor-1: faster, reliable and accurate measurements with a new electrochemical impedance spectroscopy (EIS) based biosensor system

Sonja A. Šelemetjev

Determination of antithyroglobulin antibodies concentration in human serum using Quartz Crystal Microbalance sensors

Zoltán Villányi

Protein homeostasis is maintained by the gene expression circuitry

Elvira Vukašinović

The effect of dietary cadmium on *Ostrinia nubilalis* (Hbn.) larval development rate and antioxidative gene expression

Veronika Zámbó

Testing the potential rate-limiting role of electron transfer proteins in fatty acid desaturation

Poster Session 2 – Biomedicine

Suzana Jovanović-Šanta

Flow cytometry analysis of MCF-7 breast cancer cells treated with 17-substituted androstane derivatives

Tamara Antonić

HB-EGF in high risk pregnancy

Lena Arizanović

Prevalence of factor V G1691A (Leiden), factor II G20210A, MTHFR C677T and PAI-1 4G/5G gene variants in selected population of Serbian women

Neda Djedović

Molecular mechanisms of ethyl pyruvate tolerogenic effects on dendritic cells

Sanja Erceg

Adiponectin and resistin gene variations and risk for colorectal carcinoma

Ljubica Gavrilović

Adrenal asymmetry in expression of catecholamine synthesizing enzyme in chronically stressed rats

Jelena Janać

The association between high-density lipoproteins characteristics and hepatic steatosis index

Ivan Koprivica

Suppresion of type 1 diabetes in mice by oral treatment with ATRA- and TGF-β-loaded microparticles

Milica Lazarević

The effect of H_2S donor GYY4137 on T cells in experimental autoimmune encephalomyelitis

Marija Mihajlović

Gender-related differences in IGF-1 concentration in patients with colorectal cancer and healthy individuals

Dejan Miljković

Ki-67 proliferation index and expression of vimentin in three different continuous cell lines

Radmila Miljković

Prophylactic treatment by recombinant banana lectin affects innate immune response in TNBS induced colitis

Goran Miljuš

Glycosylation of the human serum transferrin as a biomarker of healthy ageing

Marina Nikolić

Fructose consumption affects glucocorticoid receptor signaling and increases lipogenesis in the liver of young female rats

Ana Ninić

Association of fatty liver index with obstructive sleep apnea

Ljubica Gavrilović

Chronic restraint stress changes catecholaminergic turnover in rat hippocampus

Jovan K. Popović

The anticancer effect of folate, B12 and glucose metabolism inhibiting non-oncologic drugs on animal model

Jovan K. Popović

Immunohistochemical assesment of folate and B12 inhibiting non-oncologic drugs influence on cancer

Isidora Protić-Rosić

Modulation of peritonal murine macrophages functional characteristics by Bet v 1-BanLec chimera

Jovana Rajić

The capability of different TGF-β isoforms to induce EMT in human conjunctival epithelial cells

Aleksandar Stojsavljević

The potential role of lead and selenium in pathogenesis of colloid goiter disease

Aleksandra Vilotić

Differential secretion of MIF in normal and transformed human trophoblast cell lines

Sanja Vujčić

Oxidized LDL in obese children and children with type 1 diabetes mellitus

Poster Session 3 – Enzymology, Bioinformatics and Biotechnology

Ana Marija Balaž

Characterization of recombinant *Phanerochaete chrysosporium* cellobiose dehydrogenase mutants with increased oxidative stability from *Pichia pastoris* KM71H strain

Petra Bankó

Investigation of the role of human HDAC enzymes in histone modification

Luka M. Breberina

Anion-π interactions in phycocyanin interfaces: a computational analysis

Milica Crnoglavac

Purification and characterization of α-glucosidase from *Saccaromyces cerevisiae* heterologously expressed in periplasmic and intracellular space of *E. coli*

Nevena Djukić

Identification of phytic acid by hydrolysis in the presence of phytase and alkaline phosphatase in oat seed

Yaraslau U. Dzichenka

Structural insights into ligand recognition of human CYP7 enzymes

Nikola Gligorijević

Quantitation of the active alpha-2-macroglobulin by trypsin protease zymography

Béla Gyurcsik

Metal ions as regulatory elements of artificial DNA cleaving enzymes

Safija Herenda

Inhibitory effect of diclofenac to activity of catalase in in vitro conditions

Karla Ilić Đurđić

Improvement in azo dyes degradation by saturation mutagenesis of lignin peroxidase catalytic tryptophan environment

Lidija Izrael Živković

Responses of *Pseudomonas aeruginosa* san ai to nanoceria

Kristina Joksimović

Bacillus sp. isolated from Japanese food Nattō

Brigitta M. Kállai

Modified vectors for wheat germ-based cell-free protein expression

Nevena Marković

Immobilized ω-transaminase ArRMut11 for the synthesis of amino-steroids

Ana Medić

Genomic and proteomic studies of the biodegradation of 2,6-di-tert-butylphenol by *P. aeruginosa* san ai

Szilvia Krisztina Nagy

Epigenetic enzyme screening assay with *in vitro* reconstituted human chromatin

Sandra Oloketuyi

Bacterial surface display of nanobodies against cancer and toxic micoalgal cells

Krisztina Percze

Amplification of aptamer libraries using a modified nucleotide

Michail A. Shapira

In silico comparative analysis of cholesterol oxidases

Marija Stanišić

Dopamine-modified pectins for laccase induced hydrogel formation and immobilization

Stefan N. Stojanović

Recombinant expression and purification of yeast Frq1 protein

Tamás Mészáros

Selection of human haptoglobin alpha specific aptamers

Saša Vatić

The effect of buffer composition and nonionic surfactants on trypsin cold stability

Poster Session 4 – Food Science and Ethnopharmacology

Milena Rašeta

Antioxidant potential of autochtonous fungus *Laetiporus sulphureus* from Balkan region

Tatjana Majkić

Cabernet Sauvignon wine as acetylcholinesterase inhibitor

Danijela Kojić

Resveratrol administration increases antioxidative capacity of honey bees under stress induced by H_2O_2

Miljan R. Bigović

Determination of proteins and carbohydrates in Igalo bay peloid (Montenegro)

Milena Rašeta

Antioxidant activity of four extracts of *Fomes fomentarius* from Bosnia and Herzegovina

Jovana Drljača

Karnozin EXTRA® alters mitochondrial respiration via inhibition the activity of complex II

Dragica Gajić

Chokeberry (*Aronia melanocarpa*) fruit extract modulates mouse immune response *in vivo* and *in vitro*

Aleksandra Jovanović Galović

Molecular effects of plant extracts: Three immunochemical methods in two examples

Desimir Knežević

Composition of gliadin proteins in bread wheat genotypes

Milica Kojadinović

Interactions of ellagic acid metabolites with human and bovine serum albumin by fluorescence quenching of protein intrinsic fluorescence

Jovan Luković

Antitumor effect of the chalcone analogue, (E) -1- (4-ethoxy-3-methoxyphenyl) -5- methylhex-1-en-3-one on HCT-116 cells

Tatjana Majkić

Plantago lanceolata L. effects gene expression of enzymes involved in prostaglandin E2 production

Stefan M. Marković

Impact of high temperature on the accumulation of eEF1A in different cereal varieties

Vladimir Mihailović

Anti-inflammatory activity and cytotoxicity of *Gentiana asclepiadea* L. extracts

Ana B. Miltojević

Influence of dose size on the metabolism of methyl and isopropyl N-methylanthranilates

Milena Milutinović

Proapoptotic activity of *Gentiana punctata* L. on colorectal cancer cells

Diandra Pintać

Analyzing biological and chemical properties of Turkish and instant coffees

Diandra Pintać

Comparing antioxidant and chemical properties of black and green tea

Tamara Popović

PON1 plasma activities in aging after fish oil supplementation

Farkas Sarnyai

Effect of dietary trans fatty acids on ceramide and diglyceride levels in rat insulinoma cells

Aleksandra Milenković

The effect of the extraction techniques on the antioxidant and antimicrobial activity of ethanolic extracts from sour cherry (*Prunus cerasus* L.) pedicles

Péter Szelényi

Inhibition of microsomal cortisol production – different polyphenols, different mechanisms

Nikola Srećković

Antioxidant and cytotoxic activities of rosmarinic acid-rich *Salvia pratensis* L. extracts

Speed Talks

November 14th

Marija Mihajlović
Faculty of Pharmacy, University of Belgrade, Serbia

Ana Medić
Faculty of Medicine, University of Belgrade, Serbia

Jelica Milošević
Faculty of Chemistry, University of Belgrade, Serbia

Tatjana Majkić
Faculty of Sciences, University of Novi Sad, Serbia

Aleksandar Stojsavljević
Faculty of Chemistry, University of Belgrade, Serbia

November 15th

Andrijana Nešić
Faculty of Chemistry, University of Belgrade, Serbia

Szilvia Krisztina Nagy
Semmelweis University, Hungary

Ana Penezić
Institute for the Application of Nuclear Energy - INEP, University of Belgrade, Serbia

Milena Milutinović
Faculty of Science, University of Kragujevac, Serbia

Milica Lazarević
Institute for Biological Research “Siniša Stanković”, University of Belgrade, Serbia

November 16th

Nikola Srećković
Faculty of Science, University of Kragujevac, Serbia

Karla Ilić-Đurđić
Faculty of Chemistry, University of Belgrade, Serbia

Jovana Drljača
Faculty of Medicine, University of Novi Sad, Serbia

Aleksandra Vilotić
Institute for the Application of Nuclear Energy - INEP, University of Belgrade, Serbia

Zoltan Villanyi
University of Szeged, Hungary

Foreword

Dear Colleagues

Welcome to the 9th Conference of the Serbian Biochemical Society, entitled "*Diversity in Biochemistry*".

This year, we have a three day conference with rich program, strong support of FEBS, and proliferated collaboration with FEBS3+ countries (Croatia, Hungary, Slovenia, and Serbia). We are getting bigger and in shape for the next FEBS3+ Meeting. I hope that you will enjoy lectures and poster sections at the conference that represents a blueprint of current biochemical and life science research in Serbia and the region.

I would like to express my gratitude to the members of the Scientific Board who suggested lecturers, to all respected colleagues who accepted the invitation, and particularly to all our dear guests that are visiting Belgrade for the first time.

Editor of the Proceedings
Ivan Spasojević

Plenary Lectures

Proteins that sense cellular environments – examples and implications

Snežana Djordjević

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The first step in the process of signal perception and transduction involves interaction between a stimulus and the specific protein that has the capacity to recognise the stimulus and to translate the interaction to the physical manifestation of a signal. The physical manifestation of the signal involves what is commonly referred to as a 'protein conformational change' that results in a change in a conformational equilibrium of the proteins that perceive the stimuli. Reflecting on our work I will describe two specific examples of stimuli perception and signal transduction mechanisms, one relating to protein AioX that is found in a prokaryotic organism adapted to living in the conditions of arsenic contamination and the second example involving neuropilins - transmembrane proteins of the significance for human health.

A journey along the TIM23 complex, the major protein translocase of the mitochondrial inner membrane

Umut Günsel, Dejana Mokranjac*

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Proper structure and function of mitochondria depends on translocation of over thousand different mitochondrial proteins from the cytosol. Mitochondrial proteins carry a number of different targeting signals and are translocated into mitochondria by a number of different protein translocases. Here, we provide an overview of mitochondrial protein translocation pathways and focus on the TIM23 complex, the major protein translocase of the mitochondrial inner membrane.

Invited Lectures

Routine and novel methods for isolation of extracellular vesicles

Milica Popović

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Extracellular vesicles (EV) play an important role in many physiological and pathological processes. Three main classes of EV are recognizes, based on their biogenesis: exosomes, microvesicles and apoptotic bodies. Exosomes are extracellular-vesicles of 30 to 150 nm found in many bodily fluids (blood, urine, milk, cerebrospinal fluid, etc.). Due to their cellular origin and the role in physiological and pathological processes, exosomes present in body fluids are considered a unique source of non-invasive and clinically relevant biomarkers. Analysis of exosomes can provide an insight into the state of the parent-cell from which they originated. However, there is a great heterogeneity in methodologies used for exosome purification affecting results of downstream analysis. Most commonly used methods for purification based on ultracentrifugation (UC), ultrafiltration (UF) and precipitation are hard to standardize leading to confronting and misleading results of the downstream analysis, especially when highly-sensitive techniques such as mass-spectrometry are used. Furthermore, loss of certain fractions or damage of EVs can lead to loss in obtained protein and RNA profile. Consequently, there is an emerging need in obtaining a consensus protocols for exosome isolation and identification of specific sub-populations. This manuscript will critically review most commonly used techniques for EV purification such as UC, UF, size-exclusion, precipitation and immunoaffinity (IA). We will also review use of nano-antibodies for development of novel IA protocols and identification of new EV biomarkers.

The role of proprotein convertase subtilisin/kexin type 9 in atherosclerosis

Jelena Vekić[1*], Dragana Bojanin[2], Vesna Spasojević-Kalimanovska[1]

[1]*Department of Medical Biochemistry, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia*
[2]*Department for Clinical Chemistry and Hematology, Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić", Belgrade, Serbia*

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Dyslipidemia is one of the predominant causes of atherosclerosis and cardiovascular diseases (CVD) development [1]. Accordingly, lifestyles approaches and therapeutic targeting of low-density lipoprotein (LDL)-cholesterol remain the main strategies for CVD prevention and treatment. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secretory serin-protease with important role in lipoprotein metabolism. In particular, PCSK9 promotes degradation of hepatic LDL-receptors, leading to reduced clearance of LDL particles and increased plasma LDL-cholesterol levels. To date, a large body of evidence from experimental, genetic and clinical studies indicates that PCSK9 is implicated in the development of atherosclerosis [2]. PCSK9 levels are significantly increased in patients with high cardiovascular risk, such as obesity, metabolic syndrome and diabetes, as well as in patients with CVD [3]. As the research is moving forward, additional roles of PCSK9 beyond cholesterol metabolism and atherosclerosis are discovered. In the present paper, we will discuss the current knowledge on the role of PCSK9 in atherosclerosis, its associations with cardiometabolic risk factors and provide a brief overview of recent achievements in pharmacological inhibition of PCSK9-mediated LDL-receptor degradation towards LDL-cholesterol reduction and prevention of CVD development.

Acknowledgment

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2. Shapiro MD, Tavori H, Fazio S. PCSK9: from basic science discoveries to clinical trials. Circ Res 2018;122:1420-38.

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The role of molecular markers of angiogenesis in the prediction disease in patients with breast cancer

Danijela Cvetković[1*], Aleksandar Cvetković[2,3], Srdjan Ninković[2,3], Milena Milutinović[1], Bojana Mitrović[4], Snežana Marković[1]

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[2]Department of Surgery, Faculty of Medical Sciences, University of Kragujevac
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Breast cancer is the most common malignant tumor in women around the world. It is a disease of complex etiology, characteristics and response to therapy. Oncology therapy is the most expensive form of treatment and the most part of the health budget of both developed and transition countries go to it. The progress in molecular biology had a major impact on the development of a personified approach. Our study clearly indicates that cancer causes changes in the cancer and peritumoral tissue detectable on the molecular but not at the pathohistological level. Therefore, analysis not only of cancer but also of the peritumoral tissue is very valuable, because often the changes that are very significant in the prognostic sense occur predominantly in the microenvironment of the carcinoma. Markers of angiogenesis in cancer and peritumor tissue such as MMP-9 concentration, expression of *VEGF-A, CXCL-12, HIF-1* and *iNOS* genes can serve as reliable predictors of disease outcome in patients with breast cancer, which can give useful suggestions in the choice of treatment. Using modern methods of molecular biology, a group of patients with an increased risk of metastases and recurrence can be identified, which is certainly one of the most important information on the basis of which the decision on further treatment is made.

Longitudinal distribution of apoplastic antioxidative components in maize root

Jelena Dragišić Maksimović[1*], Miloš Mojović[2], Željko Vučinić[1], Vuk Maksimović[1]

[1]*Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia*
[2]*Faculty of Physical Chemistry, University of Belgrade*

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The apoplast is a liquid- and gas-filled extracellular continuum which includes cell wall polymer networks and the external surface of the plasma membrane. The apoplastic constituents such as various organic molecules, enzymes and proteins play the major role in a wide range of physiological processes. In order to investigate apoplastic fluid, two isolation procedures were compared and critically evaluated: infiltration and/or centrifugation technique to obtain apoplastic washing fluid and filter paper strips which, based on our results, allows collecting experimental data from intact plants. Different components of the antioxidative system (enzymes, phenolics, sugars, organic acids) present in the apoplastic fluid were analyzed using different techniques. Three classes of non-enzymatic compounds (organic acids, sugars and phenolics) have been identified and quantified by HPLC. Detection of hydroxyl radicals was performed by EPR method using spin-trap DEPMPO which is capable of forming different spin-adducts with hydroxyl and superoxide anion radicals. Spectrophotometrically estimated total protein concentrations, peroxidase and superoxide dismutase specific activities, as well as their different isoforms were visually confirmed by isoelectric focusing. All presented high sensitivity techniques (HPLC-ECD, EPR), as well as electrophoresis, in combination with the filter strip method provided us a tool to study components of the antioxidative system in the apoplast of developing plant organs and their spatial-temporal changes. Such an experimental setup provides a powerful non-invasive analytical tool for studying metabolic processes occurring in the apoplast and local changes in small regions of the intact root tissue.

Molecular adaptation to high temperatures: pernisine from the archaeon *Aeropyrum pernix* K1

Kevin Hartman, Miha Bahun, Marko Šnajder, Nataša Poklar Ulrih*

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Pernisine is a subtilisin-like protease from the hyperthermophilic archaeon Aeropyrum pernix. Due to its high thermal stability and its activity in the presence of denaturants, pernisine represents a promising enzyme for use in various industrial applications. Another potentially applicable characteristic of this protease is its ability to degrade infectious prion aggregates. The production of pernisine in *A. pernix* does not provide sufficient yield for its commercial use, and alternative production strategies are hence needed. This review summarizes the biochemical and biophysical characteristics of pernisine and the progress that has been made with the production of a recombinant form of pernisine using *Escherichia coli* and *Streptomyces rimosus* as expression systems.

Engines of mutagenesis and carcinogenegesis; replication of damaged DNA

Lajos Haracska[1,2*], Lili Hegedűs[2], Lajos Pintér[3], Kata Dudás[2], Ernő Kiss[2], Li Qiuzhen[2], Mónika Mórocz[2]

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Cancer is a genetic disease caused by mutations in the DNA. Why mutations develop with a faster speed during carcinogenesis is an exciting and still open question. It is known that the genome is constantly exposed to different exogenous and endogenous DNA-damaging factors which, by generating DNA lesions, block the movement of the replication machinery since the replicative DNA polymerase in unable to insert nucleotides opposite certain DNA lesions. Stalling of the replication fork can lead to strand breaks, chromosomal rearrangements, genome instability, and eventually cancer. To rescue the stalled replication fork, DNA damage tolerance pathways have evolved facilitating mechanisms that help to achieve replication across the lesion. Such DNA damage tolerance pathways are translesion synthesis and template switching. During translesion synthesis, specialized DNA polymerases can insert nucleotides opposite the damaged bases on the template [1], while in template switching the blocked primer is switched from the damaged template to the newly replicated nascent strand of the sister duplex, which then serves as a template for DNA synthesis [2]. Our research now focuses on the regulatory mechanisms[3] of the above DNA damage tolerance pathways, by which we aim to reveal the molecular origins of mutagenesis. In particular, we investigate the following questions: What are the molecular mechanisms of chromosomal rearrangements and the formation of point mutations? Why do we observe increased genome instability during carcinogenesis? What is the role of the DNA damage tolerance genes in cancer suppression? Our recent studies have described new players and pathways of DNA damage tolerance that can bring us closer to answering the above questions. To further characterise these players, we reconstitute DNA lesion bypass using purified proteins and apply single-cell-based methods such as DNA-fiber and comet assays. In addition to shedding more light on DNA damage tolerance, our research has the potential to discover new tumour suppressors and provide novel cancer therapeutic targets.

Acknowledgements

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Detoxification of tabun-exposed mice by an acetylcholinesterase mutant assisted with a novel pyridinium aldoxime

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Nerve agents, like tabun, are covalent inhibitors of acetylcholinesterase (AChE), an essential enzyme in neurotransmission whose inhibition may lead to death. The currently used therapy, consisting of an anticholinergic drug and an oxime as the reactivator of inhibited AChE is particularly ineffective in cases of tabun exposure, so finding an optimal reactivator is an ongoing effort. Click-chemistry, utilizing Cu (I)-catalyzed azide-alkyne cycloaddition of a library of small molecule building blocks, has made possible a rapid synthesis of a variety of new oximes. Among the new oximes tested in recent studies as reactivators of tabun-inhibited choline binding site AChE mutants, oxime 5B, a lengthened alkylchain congener of the standard oxime 2-PAM, stood out as a candidate for tabun ex vivo scavenging when paired with the Y337A mutant of AChE. Herein, we pursued the antidotal in vivo detoxification of tabun-exposed mice by assembling oxime-assisted catalytic scavenging using the mutant combined with oxime 5B. Although the antidotal treatment showed drawbacks, our findings offer a platform for further development of more potent means of counteracting tabun and related phosphoramidate exposure.

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Elongation factors Tu and 1A: multifunctional proteins involved in plant heat tolerance

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Protein synthesis elongation factors EF-Tu and eEF1A (EFs) are highly conserved and abundant GTPases in living cells, with a major role in transporting the aminoacyl-tRNA complex to the ribosome during translation. EFs are also considered multifunctional proteins with numerous noncanonical activities. EFs possess chaperone activity in preventing protein aggregation, interact with misfolded newly synthesized polypeptides and possibly direct them to the proteasome, and participate in viral replication [1]. eEF1A is also implicated in the organization of cytoskeleton and apoptosis, while EF-Tu of bacterial pathogens elicits plant innate immunity [1]. There is growing evidence that up-regulation of EFs genes by abiotic stresses may play an important role in stress responses in plants. The heat stress-induced accumulation of EFs has been detected in a number of plant species, and it correlates with the heat tolerant phenotype in maize, wheat, and potato [2]. Accumulation of EFs may enhance protein synthesis and the capability of plant cells to sustain heat stress. Besides, the proposed EFs role in the quality control of newly synthesized proteins and chaperone activity in preventing protein thermal aggregation and inactivation might be important in alleviating negative effects of high temperature. Taken together, these multifunctional proteins may represent coordinators of the cellular processes in stressed plants, for instance, protein synthesis with recognition and repair/degradation of misfolded proteins.

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How to use educational technology to make education better - Not just different or entertaining!

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Educational Technologies (EdTech) have an important potential to improve the learning and teaching both in- and outside the classroom. EdTech tools can potentially make the education process more meaningful, more engaging, more interesting, more personalized and more flexible. There may be many reasons (change in school policy, pressure from the authorities, observing models of efficient use models, etc) and facilitator factors (support from the school, collaboration among faculty, etc) to implement EdTech as well as there are barriers (lack of technological tools, etc.) and drawbacks (technological competence, established teaching practices, etc). In practice, educators are also faced with the challenges in designing or integrating EdTech in a pedagogically meaningful way. Most of the time, educators struggle to effectively implement EdTech in their curriculum, however we do see numerous examples of EdTech implementations without a clear aim, vision, assessment and/or positive learning outcome. This article will start with the definition (what it is not and what it is) of EdTech and continue with discussing the 'why' (to integrate and we keep away) and 'how' (to choose and to implement) components of integration. Finally, it will close up with giving some take home messages and tips to integrate EdTEch successfully.

How FEBS supports young scientists´ careers

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The Federation of European Biochemical Societies (FEBS) brings together molecular life scientists in Europe and neighbouring regions. There are many activities of FEBS targeted to young scientists with the aim of promoting their careers. This article provides a brief overview of such FEBS initiatives.

Gonadotropin-releasing hormone regulated transcription of gonadotropin subunit genes

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Two gonadotropins, luteinizing hormone and follicle-stimulating hormone, are synthetized and secreted by anterior pituitary gonadotropes and act on the gonads, controlling gametogenesis and sex hormone production. These hormones are glycoprotein polypeptides, composed of specific beta subunits and a common, alpha subunit. Both transcription and secretion of gonadotropins are regulated by gonadotropin-releasing hormone (GnRH), which is produced by small number of hypothalamic neurons within the preoptic area and mediobasal hypothalamus. GnRH is released and is reaching the pituitary in pulses, a pattern of secretion that is crucial for the proper reproductive functions. This mini review covers mechanisms of transcriptional control of gonadotropin subunit genes by GnRH, predominantly focusing on in vivo experiments with mice and rats and in vitro experiments using primary pituitary cell cultures and immortalized pituitary cell lines derived from these species. We also provide an overview of the promoter regions of gonadotropin genes and major transcription factors involved in GnRH-driven expression of gonadotropin subunit genes.

Metallomics and mass spectrometry for drug development: employing ICP MS and MALDI TOF MS for assessing protein-drug interactions

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Ever since the discovery of anticancer drug cisplatin, the field of inorganic medicinal chemistry has been expanding. A plethora of metal-based compounds (Pt, Ru, Au, Pd, Cu) containing organic or inorganic ligands (NH_3, Cl, DMSO, η^6-arene, imidazole, indazole, terpyridine, phosphoadamantane) has been developed with the aim to find new and improved anticancer therapeutics, which would have less side-effects and to overcome tumour resistance [1]. The experimental tasks for the medicinal inorganic chemistry community are: how these compounds enter a living cell, how they distribute inside a cell, what are their intracellular targets, how they behave in the circulation (free vs protein-bound fraction), what are their target tissues, what biomolecules are responsible for their transport to biological targets. All of these questions have been recently addressed with the aid of different mass spectrometry (MS) techniques. Inductively coupled plasma MS is a highly sensitive method for determining the metal content in biological samples. Matrix-assisted laser desorpton/ionisation and electrospray MS are used to characterise the adducts between metallodrugs and proteins. MS methods provide information on binding kinetics, binding parameters, nature of the adducts and target sequences in proteins. This review deals with the most interesting results obtained by the most influential mass spectrometrists working in the field of inorganic biochemistry.

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An overview of biological activities of less known wild onions (genus *Allium* sect. *Codonoprasum*)

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Cultivated forms of *Allium* species, such as onion, garlic, leek and shalot, are highly present in human diet and are very reputable medicinal plants. Otherwise, wild onions, esspecially species from *Codonoprasum* section, are much less researched and known, even though they are present in local diet and traditional medicine. Therefore, in recent years, chemical composition and biological activities of several species of onions from sect. *Codonoprasum* were intensively studied in order to estimate their potential for application in medicine. LC-MS/MS analysis of phenolic compounds showed that species from sect. *Codonoprasum* are rich in phenolics, particularly in quercetin glycosides and kaempferol 3-*O*-glucoside [1-3]. *A. flavum* expressed high antioxidant activity in common *in vitro* assays and high anti-inflammatory activity in human platelets [1]. *A. flavum* and *A. melanantherum* exhibited great antigenotoxic effect in comet assay [2]. *A. flavum* and *A. carinatum* drastically improved activity of doxorubicin (Dox) against cancer cells[3]. In zebrafish model, *A. flavum* and *A. carinatum* expressed high anti-angiogenic activity and protective effect against Dox-caused cardiac dysfunction and neutropenia [3]. The results indicate multiple beneficial pharmacological activities of wild *Allium* species.

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In silico methodologies in biochemistry

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Traditional research tools such as *in vivo* and *in vitro* models have consistently used by scientists to test biochemistry hypotheses. Increasingly over the last decade however we have seen that computational (*in silico*) methods have been developed and applied to biochemistry hypothesis development and testing. *In silico* methods are aimed to obtain an appreciation of the quantitative aspects of analyzing scientific (big) data either stored in large data databases or generated by sophisticated modeling and simulation tools; gained a basic understanding of applying various bioinformatics methods to large biological data sets and the realization of the potential of scientific computing for the study of the behavior of biological systems, in particular large biological macromolecules. This paper presents short review of methods used in biochemistry studies like genome-wide mapping of protein-DNA interaction, proteomics-based bioinformatics, and high-throughput mapping of protein-protein interaction networks. Commonly employed modeling and simulation techniques are also presented and they include molecular dynamics, Monte Carlo and Langevin (stochastic, Brownian) dynamics, continuum electrostatics, statistical thermodynamics, protein modeling techniques, protein-ligand docking, protein-ligand affinity calculations and the computer simulation of the protein folding process and enzyme action [1–3].

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Sponsored Lectures

When proteins matter – beyond interactions and protein stability

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In 1947 Arne fon Thiselius and The Theodor Svedberg, two Nobel prize winners from Institute of Biochemistry of Uppsala University, Sweden, initiated creation of LKB Produktor AB company, first company truly devoted with Institute of Biochemistry to development and production of instruments and technologies for life sciences. Electrophoresis, chromatography, high performance liquid chromatography, precision microcalorimetry, ultramicrotomy, very high speed ultracentrifugation and many other developments are firmly bound to these two very connected institutions. In 1982 LKB Produktor AB created first biocompatible High Performance Liquid Chromatography system designated to work with proteins and biological samples. It was made of titanium alloy with no Fe, Cr and other heavy metals to leach with specially designed pumps and valves to avoid air trapping. LKB HPLC system became paradigm of protein analytics. At the same time Pharmacia AB, Uppsala, Sweden, put on the market their Fast Performance Liquid Chromatography system (FPLC) which became paradigm of protein isolation. In 1994 process of merging these two companies was completed and, as one of many spin off's, the separate, privatized company was created that still exists today: LKB Vertrirebs Ges.m.b.H. in Vienna, Austria with the special status of distributor, service and supporting provider for South East Europe. After several further merges and buy-offs what was LKB/Pharmacia, today this is GEHC Lifesciences. One of their first products was creation of the line of automatic, software supported chromatographic systems named AKTA, based on decades of engineering and expert knowledge from time of LKB HPLC and Pharmacia FPLC systems. Today we have a newcomer: AKTA go system designed to help people in busy lab and cold room environments, small, powerful and fully equipped, but simple to use as the name speaks. Results of chromatography separations must be analysed by electrophoresis, western blotting and by other means were biomolecular imaging and quantitative analysis are of the great importance. Another newcomer is Amersham™ ImageQuant™ 800 biomolecular imager with an exceptional Fujinon F 0.74 lens specially

designed for this imager with ability to read from UV to long infra-red spectrum. System is designed to even read HCP DIBE blots and microplates for routine HCP testing presence.

Posters

Antioxidant potential of autochtonous fungus *Laetiporus sulphureus* from Balkan region

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Fungal species contain a variety complex compounds derived from secondary metabolism (phenolic compounds, polyketides, triterpenoids, steroids) which are specific to each fungal species and strain. Edible species, *L. sulphureus* (Bull.) Murrill (1920) also known as „chicken of the woods" has been used for centuries in traditional medicine in many European countries because of its antipyretic, antitussive, antireumatic as well as antioxidant activities [1]. Therefore, the use of foods reach in antioxidants, may be the most relevant factor in the prevention of oxidative stress related diseases [2]. The main goal of this study was to determined the antioxidant potential and total phenolic content (TP) in hot water (H_2O), ethanolic (70% EtOH), chloroformic ($CHCl_3$) and phenolic extracts (80% MeOH) by spectroscopic analysis. In order to evaluate antioxidant potential, reducing power (FRAP) assay was undertaken together with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (2,2'-azino-bis) ABTS radical scavenging assay. All extracts showed significant antioxidant potential which is comparable with synthetic antioxidant propyl gallate (PG). The highest DPPH scavenging activity was expressed in the ethanolic extracts (IC_{50}=71.79 ± 1.35 μg/mL) as well as in ABTS (76.86 ± 1.74 mg TE/g d.w.) and FRAP (151.50 ± 0.17 mg AAE/g d.w.) assay. In term of TP, the highest content was determined also in the EtOH extracts (124.95 ± 1.00 μg/mL) and it was in good correlation with determined antioxidant activities. In conclusion, obtained results indicate significant antioxidant potential of ethanol extracts of *L. sulphureus* and support its use as food supplement with healthy and nutritional benefits.

Acknowledgements

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Flow cytometry analysis of MCF-7 breast cancer cells treated with 17-substituted androstane derivatives

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Breast cancer is among the most widespread diseases with a fatal outcome. Estrogen receptor positive breast cancers could be treated by both hormonal and cytostatic therapies. However, in patients in reproductive period hormonal therapy is not always effective. On the other hand, chemotherapy usually causes serious side effects. These facts, as well as the fact that some steroidal compounds are known for their anticancer properties, prompted us to test novel modified steroids for their anticancer activity in order to recognize potential new therapeutics with low side effects. Compounds with high potential for growth inhibition of MCF-7 breast cancer cells (compounds with low IC_{50} concentrations) and good selectivity were tested for the mechanism underlying this antiproliferative effect. After the treatment with equitoxic concentrations of selected steroids, specific dyes were added and cells were analyzed by flow cytometry. This tool was used for counting cells which underwent some specific change, caused by treatment with steroids. Some of the 17-substituted androstane derivatives induced apoptosis, while some changed mitochondrial membrane potential (MMP) of treated cells. Cell cycle of treated cells was not influenced highly. The highest impact on treated cancer cells steroidal compounds exerted via inducing production of reactive oxygen species (ROS). For couple of tested steroids connection between production of ROS and change in MMP was established, with no clear evidence about type of cell death or cell cycle arrest for 72 h treatment period. Accordingly, further search for a mechanism of cell growth inhibition is needed.

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Cabernet Sauvignon wine as acetylcholinesterase inhibitor

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Alzheimer disease (AD) is a neurodegenerative disorder manifested through memory loss, cognitive dysfunction, changes in behaviour, etc. Today, there is no drug which could cure AD, and available remedies can only alleviate symptoms. Great challenge of modern medicine is a discovery of new drugs, capable to combat or halt dementia. One of the most promising approaches in the treatment of AD is inhibition of acetylcholinesterase (AchE), an enzyme involved in progression of AD.[1] Numerous reports testify that Mediterranean diet, rich in polyphenols, could delay disease connected with ageing, and, among others, neurodegenerative disease. Therefore, the aim of this study was to examine the potential of five Cabernet Sauvignon wine extracts to inhibit AChE. The Ellman`s spectrophotometric method, adapted to 96-well microplates, was used to determine the AChE inhibitory activity. Besides, total content of phenols, flavonoids, tannins and anthocyanins were determined spectrophotometrically in all samples.[2] Statistically significant differences were found between samples activities (IC_{50} values ranged from 0.56 to 1.94 mg/mL), but, in comparison to physostigmine, a well known AChE inhibitor, extracts exhibited moderate neuroprotective potential. The total phenols, flavonoids and tannin contents of the samples exhibited some correlation with examined activity. Obtained results suggest that examined Cabernet Sauvignon wine extracts could be regarded as potential good neuroprotective agents. Also, they support the further investigation to identify exact compounds of the wine which are responsible for the substantial anti-AchE activity.

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HB-EGF in high risk pregnancy

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Heparin-binding epidermal growth factor - like growth factor (HBEGF) is believed to be one of the factors which regulate trophoblast differentiation, making it a candidate risk factor for preeclampsia development. HBEGF, primarly related to the earliest stage of pregnancy, is thought to be decreased in preeclampsia[1]. Eighty pregnant women with high risk pregnancy were included in the study to examine whether HBEGF concentrations are changed throughout the gestation. Inclusion criteria were chosen in accordance to the NICE Quality standard (QS35). Blood samples were obtained at the end of each trimester and HBEGF concentration was evaluated using ELISA kit. HBEGF concentrations were classified into two groups – lower than the limit of quantification and as measurable concentration and were further evaluated as categorical variable. Cochrane's Q test showed statistically significant difference in the proportion of pregnant women with measurable HBEGF concentrations during pregnancy ($p<0.001$). Using McNemar's test we noticed significantly higher proportion of pregnant women with measurable HBEGF concentrations in second ($p<0.001$) and third ($p<0.001$) compared to the first trimester, while the difference in proportion was not significant when comparing second to third trimester ($p<0.134$), indicating HBEGF could be lower in the early stage of gestation. However, we failed to show the difference in proportion of low HBEGF values in women who developed preeclampsia compared to those without preeclampsia.

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Prevalence of factor V G1691A (Leiden), factor II G20210A, MTHFR C677T and PAI-1 4G/5G gene variants in selected population of Serbian women

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Mutations in genes for coagulation factor V, factor II prothrombin, methylenetetrahydrofolate reductase (MTHFR), and plasminogen activator inhibitor-1 (PAI-1) represent well-known genetic risk factors for thrombophilia, that are increasingly recognized as possible causes of complications in women reproductive health. In this study, the prevalence of FV G1691A, FII G20210A, MTHFR C677T, and PAI-1 4G/5G gene variants was tested in a sample of Caucasian females of reproductive age. The starting materials for genotyping were either anticoagulated whole blood or buccal swabs taken from 584 subjects from Serbia aged 21-44. Genotyping of isolated genomic DNA of mentioned gene variants was done by multiplex PCR followed by capillary electrophoresis. No mutations (i.e. wild-type alleles for all four tested genes) were found in only 43 subjects who represent 7.4% of total sample size. Detected FV G1691A and FII G20210A carrier rate was 5.3% for both mutations, with allele frequencies of 0.026 (FV 1691A) and 0.027 (FII 20210A); the heterozygous genotype for both factors was detected in three women (0.5%). The prevalence of the heterozygous MTHFR C677T and PAI-1 4G/5G genotype was 43.2% and 47.8%, but the measured corresponding homozygotes mutation rate was 15.0% and 33.0%, respectively. Calculated MTHFR 677T and PAI-1 4G allele frequencies were 0.363 and 0.571. In conclusion, our study of the largest so far known sample of female subjects demonstrates slightly higher values for the test parameters in comparison with the available, subject-matched literature data acquired on smaller samples of tested subjects.

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Characterization of recombinant *Phanerochaete chrysosporium* cellobiose dehydrogenase mutants with increased oxidative stability from *Pichia pastoris* KM71H strain

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Phanerochaete chrysosporium is a white rot fungi and it has been known to secrete flavocytochrome enzyme cellobiose dehydrogenase (CDH, EC 1.1.99.18) which contains two domains, a flavine domain and cytochrome domain. Flavine domain contains FAD as prostetic group and its catalytically active domain, whereas cytochrome domain serves as electrone acceptor. Cellobiose and lactose, as well as other β – 1,4 – linked disaccharides and oligosaccharides, have been oxidized by the cellobiose dehydrogenase to their corresponding lactones [1-3]. CDH can be used for constructing biosensors and therefore directed evolution has been used to produce more active and stable variants of the enzyme. Wild type CDH enzyme was expressed in *S.cerevisiae* INVSc1 cells and used for creation of saturation mutagenesis libraries at M65, M685 and M738 and screening for increased oxidative stability. More stable mutants that were found were recloned into *Pichia pastoris* KM71H strain for higher expression yield. They were afterwards, expressed in *Pichia*, purified and kineticaly characterized.

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Investigation of the role of human HDAC enzymes in histone modification

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Human histone modifying enzymes have been in the center of growing attention due to their potential in new treatment of diseases. Histones are small and alkaline proteins, present in an octameric form, composed of two copies of the four core histones (histone H2A, H2B, H3 and H4). The octamer is wrapped around by the chromosomal DNA forming the basic repeating unit of the chromatin, called nucleosome. Determining the function of histone modifiers is a challenging task, due to the vast complexity and dynamic nature of these modifications. In this work, we aim to develop an *in vitro* enzyme screening assay for quick and straightforward functional characterization of histone modifying enzymes. To achieve this, our approach is to reconstruct the human nucleosome by expressing the histones in wheat-germ based *in vitro* translation, to serve as a substrate for screening of various modifiers. As a proof of concept, we produce a set of histone modifying writers (methyl transferases, acetylases, ubiquitin ligases and kinases) and erasers (demethylases, deacetylases, deubiquitinases and phosphatases) and test known modification. I amplified histone deacetyl transferases (HDAC) from human cDNA libraries and inserted to pEU3-NII-HLICNot vector by ligation independent cloning[1]. Recently, I successfully cloned the genes for HDAC2, HDAC3 and HDAC11, which will be expressed by *in vitro* translation and used for chromatin modification enzyme assays. The modifications will be tracked by the state-of-the-art mass spectrometry (Q Exactive plus Orbitrap). We aim is to reveal new modification processes and ultimately, our method can be an essential tool to examine epigenetic modifications *in vitro.*

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Resveratrol administration increases antioxidative capacity of honey bees under stress induced by H_2O_2

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Honey bees belong to the world's most important pollinator, but recently a decline in managed colonies has been reported probably due to influence of various environmental and anthropogenic factors[1]. Resveratrol is a plant polyphenol with well reported antioxidant activity and effects of life extension on diverse organisms[2]. In present study, to test antioxidative effects of resveratrol on honey bee under stress condition induced by H_2O_2, we supplemented the diet with resveratrol at concentration of 50 µM during ten days. We measured the antioxidant capacity by FRAP assay and contents of protein thiol groups, reduced glutathione (GSH) and malondialdehyde (MDA), as markers of oxidative status, in stressed groups with or without resveratrol supplementation. Results showed that resveratrol supplemented honey bees had a significantly reduced MDA level and increased antioxidative capacity compared with unsupplemented control. Also contents of protein thiols and GSH increased (protein thiols significantly). Our results indicated beneficial effects of resveratrol on oxidative status of honey bees under stress condition. Based on our results the potential application of resveratrol supplementation in beekeeping practice could be considered.

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Optimization of ligand binding assay in yeast for identification of phytoestrogens with affinity for estrogen receptor β

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Phytoestrogens are compounds derived from plants, structurally similar to female steroid hormones, estrogens, in mammals. These naturally occurring compounds play an important role in the treatment of hormone-dependent disorders characterized by low estrogen level, such as menopause and osteoporosis. Additionally, protective effect of some isoflavones on cardiovascular system was recognized. Due to their beneficial effects on health, development of new assays for identification of phytoestrogens with high affinity for estrogen receptor is of great importance. In this study, we tested binding of selected phytoestrogens for estrogen receptor β (ERβ) using optimized fluorescent cell assay in yeast. Use of this non-transcriptional assay in evaluation of relative binding affinities of synthesized steroidal compounds for steroid receptors was previously proven. Ligand binding domain of ERβ fused with yellow fluorescent protein (YFP) was expressed in *Saccharomyces cerevisiae* FY250 strain. Following ligand-induced dimerization of steroid receptor, fluorescence resonance energy transfer between two YFP molecules occures leading to measurable fluorescence signal. Fluorescence intensity was evaluated by cell fluorimetry and fluorescence microscopy, making this system very sensitive and reliable. All tested phytoestrogen standards (biochanin A, genistein, daidzein, formononetin and glycitein) showed strong affinity for ERβ ligand binding domain comparable to that of natural ligand estradiol. Our results suggest that this highly specific assay can be useful tool for quick screening of plant extracts in order to find those with potential estrogenic properties.

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Determination of proteins and carbohydrates in Igalo bay peloid (Montenegro)

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Thermal mud found at the coast of Igalo has a long history of therapeutic and cosmetic use. Known for a number of therapeutic properties it is a resort for healing, cosmetic and/or aesthetic applications and due to its closeness of the sea an extremely valuable natural asset [1-3]. The organic composition ofthe Igalo peloid has not been investigated or determined. In our work we examined the content and composition of carbohydrates and proteins in the Igalo peloid. For the purpose of this study, different analytical techniques were applied to the collected peloid, including extraction, electrophoresis and chromatographic analysis. Igalo peloid is mildly acidic with close to 9% (w/w) of total organic matter. Total percent of nitrogen was determined to be 0.097 ± 0,004%, which among other included fourteen amino acids and protein content of 3.71 ± 0.01 μg/g of wet mass of peloid. The protein content of Igalo peloid was determined after extraction of the material with phosphate buffer saline. Protein composition was further analyzed using SDS-PAGE. By chromatographic analysis we were able to detect and determine four monosaccharides The most abundant monosaccharide present in peloid is arabinose. Monosaccharides identified in the peloid can be found in naturally occurring biopolymers and therefore were most probably derived from a mixture of phytoplankton, marine bacteria and terrestrial plants. Our results suggest that the presence of many biologically active organic compound may be beneficial for the balneological value of Igalo peloid.

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Anion-π interactions in phycocyanin interfaces: a computational analysis

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We investigated 321 possible anion-π interactions in a data set consisting of different subunit interfaces in 20 phycocyanins PDB structures. We observed that phycocyanobilin tetrapyrrole chromophore is capable of forming anion-π interactions only as an anion. It was found that Tyr is the most common aromatic donor. Although presented in the examined set of interfaces, Trp does not take part in anion-π interactions. Asp-Tyr is the most common anion-π pair, while Glu-His pair does not exist in our data set. Distance examination revealed that anion-π interactions between amino acid residues appear in the range of 3-7 Å, with the average value around 5 Å. The angle between carboxylate and aromatic ring points preference toward higher values, with a peak at 90° and average value around 66°. Interestingly, much less represented anion-π contacts including chromophore show higher values of distance and lower values of this angle. *Ab initio* calculations revealed that interaction energies lay in the range from +0.3 to –14 kcal mol^{-1}, with generally much higher values for anion-π interaction pairs between amino acid residues compared to the chromophore including interactions. The most common (>50%) anion-π residue in the stabilization protein centers is Phe, while Glu and His are not presented at all. Anion-π interacting residues have high average conservation score of 7.7, which is especially pronounced for anion residues. The highest conservation score is observed for Asp and the lowest for Phe. Anion-π interacting residues show a preference for buried regions. Almost half of the residues involved in anion-π interactions are also part of hot-spot regions, but only Asp, Phe, and Tyr. Further, in approximately one-fifth of anion-π interaction pairs, both of the residues come from the same hot-spot region and these are exclusively Asp-Tyr contacts. To conclude, a high percentage of anion-π interacting pairs in stabilization centers, their high presence in hot-spot regions and high conservation score, together with the favorable energy profile, imply that these interactions might have a significant role in the stability of phycocyanin oligomers.

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Purification and characterization of α-glucosidase from *Saccaromyces cerevisiae* heterologously expressed in periplasmic and intracellular space of *E. coli*.

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α-Glucosidases (EC 3.2.1.20, α-D-glucoside glucohydrolases) are exoenzymes hydrolizing terminal glycosidic bonds and releasing α-glucose from non-reducing end of the substrate chain. These enzymes are widely distributed in microorganisms, plants, and animal tissues. Many α-glucosidases can hydrolyze not only oligosaccharides and synthetic α-glycosides containing α-glycosidic bonds but also α-glucans such as water-soluble starch and glycogen [1]. α-Glucosidases could be considered as transglucosidases because some of them catalyze both hydrolysis and transglucosylation, which can be very useful in production of different glycosylated products. In this study our goal was to express, purify, characterize α-glucosidases and compare enzymes expressed in periplasmis and intracellular space.We selected *E. coli* for expression system because of its ease of manipulation and the quantity of protein that can be obtained [2]. We used different lysis methods depending on whether the protein is expressed, in the periplasm or intracellular space. Purification was based on IMAC (immobilized metal affinity chromatography) HPLC because of His-tag presence in the expressed proteins. Steady-state kinetics parameters, pH stability, pH optimum, temperature stability and temperature optimum were determined for both form of enzymes. Besides this, advantages and disadvantages of periplasmic expression vs. intracellular expression are also discussed. We concluded that periplasmic expression has a lot of benefits like easier manipulation and cost-effectiveness of purification of the target protein.

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Comparative study of different DNA isolation methods from plants and fungus

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Isolation of Genomic DNA is the first step in many molecular analyses today. It is required to get a high DNA yield and minimal presence of secondary metabolites. Polyphenol compounds found in plants and fungi may have negative impact on PCR, NGS and other methods [1]. For those reasons, in this study, two manual protocols, with SDS buffer[1] (protocol 1) and CTAB buffer (protocol 2) were compared with GeneJET Plant Genomic DNA Purification Mini Kit (protocol 3) [2]. For isolation, material from two plants was used: *Robinia pseudoacacia* L. and *Amorpha fruticosa* L. and one fungus: *Urnula mediterranea* (M. Carbone, Agnello & Baglivo). Absorbances from DNA pellet were measured at Biophotometer (Eppendorf) on 260/280 nm. Means of DNA concentration (C, µg/ml) and purity (P, absorbance 260/280) for protocols 1, 2 and 3 are: (a) *R. pseudoacacia* C - 14.85, 10.1, 23.46 and P - 1.1, 1.6, 1.17; (b) *A. fruticosa* C - 43.23, 36.07, 11.1 and P - 1.04, 1.13, 1.43 (c) *U. mediterranea* C - 5.27, 94.1, 3.4 and P - 1.33, 1.23, 2.12. Results showed that protocol 1 have good DNA yield from plants but have high standard error. This error could be caused by different factors, such as human, chemicals, equipment etc, suggesting that this protocol is not reliable. Protocol 2 have good concentration for fungus. When yield and purity are considered, for all tested species, protocol 3 is the most reliable. In conclusion, regarding to further analysis, protocol with CTAB buffer is recommended for high DNA yield for fungi, but isolation with Kit is recommended as the most standard one for both plants and fungi.

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Molecular mechanisms of ethyl pyruvate tolerogenic effects on dendritic cells

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Dendritic cells (DC) are professional antigen presenting cells that have a key role in regulating the immune response. Tolerogenic dendritic cells (tolDC) have immuno-regulatory properties and they are a promising prospective therapy for multiple sclerosis, as well as for other autoimmune diseases. Ethyl pyruvate (EP) is a redox analogue of dimethyl fumarate (Tecfidera), a drug for multiple sclerosis treatment. We have recently shown that EP has the ability to direct DC towards tolDC in both murine and human DC. In order to investigate mechanisms responsible for EP-imposed tolerance in DC, Nrf2 signalling pathway, HO-1 and NQO1 enzymes responsible for anti-oxidative cell protection were examined. Furthermore, activation of pro-inflammatory transcription factor NF-κB was also determined. EP was applied on days 3 and 6 during 7 days long differentiation of C57BL/6 mouse bone marrow derived immature DC (iDC) or lipopolysaccharide induced mature DC (mDC). Afterwards, immunocitochemistry staining was performed. Results have shown that the maturation of DC led to a reduction of the Nrf2 and HO-1 expression, which was successfully prevented by EP. Furthermore, the expression of NQO1 was higher in EP-treated iDC in comparison to untreated iDC. However, the expression in EP treated mDC was lower than in untreated mDC, but still higher than in iDC. EP-treated mDC had lower expression of NF-κB compared to EP-treated iDC. In conclusion, these results clearly demonstrate that EP exercises its tolerogenic potential on DC through the up-regulation of anti-oxidative signalling pathways, as well as through the inhibition of pro-inflammatory transcription factor NF-κB.

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Identification of phytic acid by hydrolysis in the presence of phytase and alkaline phosphatase in oat seed

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Mio-inositol hexafosfate (IP6), known as phytic acid or phytate in salt form, is the primary source of inositol and the most known reserve form of phosphorus in many plant tissues. It can be found in cereals, legumes, nuts and oil seeds [1]. Phytic acid content of cereals varies from 0.5 to 2.0% [2]. The aim of this study was the identification and quantitative analysis of phytic acid in ten varieties of oats (*Merkur, Minor Abed, Flamingz-kurz, Nuptiele, Simo, Prode, Pellerva, Emperor, Astor and Osmo*). For the determination of the phosphorus concentration of phytic acid, used the Megazyme method (K-PHIT). The method involves the extraction of phytic acid (IP6) and lower myo-inositol phosphate forms (IP5, IP4, IP3, IP2), using phytase. Subsequent treatment with alkaline phosphatase ensures the release of the final phosphate from myoinositol phosphate (IP1) which is relatively resistant to the action of phytase. The phosphorus released is measured spectrophotometrically. The calculation of phytic acid content assumes that the amount of phosphorus measured is exclusively released from phytic acid and that this comprises 28.2 % of phytic acid. In this study differences among ten varieties of oats is established according to the content of phosphorus and phytic acid was established. The lowest concentration of phytic acid (0.0824 g/100 g) showed the *Prode* variety. The *Astor* variety had the highest concentration of phytic acid (1.321 g/100 g). Other varieties vary in concentration of phytic acid with an average of 0.6833 %. A large amount of phytic acid in grains is a problem in nutrition for humans and animals. This acid is an antinutritient, because it binds many important minerals such as $Fe^{2+/3+}$, Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}. But, on the other side, phytic acid has many positive effects: anticancer, antioxidative, neuroprotective, hypoglycemic and antimicrobial [3]. Research on the variability of phytic acid content in cereals can contribute to the cultivators to create varieties with the optimum content of phytic acid.

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Antioxidant activity of four extracts of *Fomes fomentarius* from Bosnia and Herzegovina

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F. fomentarius (L.) Fr. is a saprophytic, perennial fungal species with wood structure which makes it inedible. The discovery of an iceberg in the Italian Alps confirmed that *F. fomentarius* was known in the territory of Europe more than 5 thousand years ago. This fungal spicies are mainly distributed in Europe, Asia and North America[1]. The use of *F. fomentarius* for medicinal purposes is a very important. It is used as medicine for diarrhea, cauterization of the wound and as painkiller. *F. fomentarius* is a important source of phenolic compounds, therefore, the aim of this study was to determine total phenol (TP) content as well as the antioxidant potential of ethanol (70% EtOH), hot water (H_2O), phenolic (80% MeOH) and chlorophorm ($CHCl_3$) extracts. Antioxidant potency was evaluated using three *in vitro* test systems[2]: DPPH (dyphenypicrylhydrazil), ABTS (2,2'-azino-bis) scavenger capacity assays and reducing power (FRAP) assay. All extracts showed potent antioxidant effect compared with PG (propyl gallate), a well-known synthetic antioxidant. Among examined extracts, ethanol extracts exhibited the powerfull antioxidant activity towards ABTS and FRAP, while DPPH scavenging capacity increased in order hot water (IC_{50}=33,06±0,71 μg/mL), ethanol (IC_{50}=3,38±0,41 μg/mL) and phenolic extract (IC_{50}=1,59±0,27 μg/mL). Determined antioxidant activities were in good correlation with the TP content (280,83±0,32 and 234,28±0,02 mg GA/g d.w. for ethanol and phenolic extracts, respectively). In conclusion, presented results highlight the antioxidant potential of analyzed *F. fomentarius* extracts, support their traditional use and may represent possible new source of pharmaceuticals in the future.

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Karnozin EXTRA® alters mitochondrial respiration via inhibition the activity of complex II

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Carnosine, an endogenous peptide, has been demonstrated to play an antitumorigenic role in certain types of cancer, suppressing glycolysis in cultured tumour cells [1,2]. Recent evidence suggests that l-carnosine can interfere with oxidative phosphorylation as well [3]. However, its underlying mechanism is unclear. The capsule of Karnozin EXTRA® (Carnomed) is a unique patented formula of l-carnosine, in combination with vitamin E, coenzyme Q10, l-carnitine, northern blueberries extract and grape seed extract. This food supplement was tested on two continuous cell lines with different energy pathways, MRC-5 (human embryo lung fibroblasts) and MCF-7 (human breast cancer cells), to evaluate its effects on mitochondrial respiration and certain mitochondrial respiratory chain complexes of the cells. Cells were treated for 24 h with different concentrations of aqueous solution of the capsule Karnozin EXTRA® corresponding to concentrations of pure l-carnosine from the capsule of 2, 5, and 10 mM. Afterwards, we investigated basal respiration of intact cells and the activities of mitochondrial respiratory chain complexes I, II and IV. All measurements were performed using the Hansatech Oxygraph+ instrument (England). Results showed that Karnozin EXTRA® exerted a significant reduction in the oxygen consumption in both cell lines in a dose-dependent manner. Moreover, the activities of mitochondrial electron transport chain complexes I, II and IV in both cell lines were compromised. The strongest inhibitory action was shown on the activity of complex II of mitochondrial electron transport chain.The present study highlights a novel role of carnosine as regulator of tested cells energy metabolism both in the anaerobic and aerobic pathways, which may give renewed impetus for its development as antitumor agent.

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Structural insights into ligand recognition of human CYP7 enzymes

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Human steroid-hydroxylases are members of the superfamily of heme-containing proteins - cytochromes P450 (CYPs). Steroid-hydroxylases are terminal oxidase enzymes in electron-transfer chains [1]. In human the enzymes are located in the endoplasmic reticulum of cells and metabolize large amount of endogenous and exogenous chemicals such as cholesterol, steroid hormones, bile acids, drugs, xenobiotics and so on [2]. *In vitro* studies followed by computer modelling simulation of interaction of modified steroids with selected human steroid-hydroxylases are presented here. We used spectrophotometric titration and reconstruction of enzyme activity to select ligands of the enzymes among androstane, estrane and bile acids derivatives. Application of molecular docking followed by MD simulation allowed us to find structural properties of human CYP7 which are crucial for ligand binding in the active site. Improvement of prediction of ligands binding based on the experimental data is the main aim of our further work. This can help in *in silico* design of drugs with high efficiency and low side effects frequency.

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Adiponectin and resistin gene variations and risk for colorectal carcinoma

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Colorectal carcinoma (CRC) is a multifactorial disease affected by different genetic and environmental factors [1]. Adipokines, such as resistin (*RETN*) produced by monocytes and macrophages and adiponectin (*ADIPOQ*) produced by adipose tissue also might be involved in CRC development [1]. The aim of our study was to examine whether there were any significant synergistic interactions of *RETN* gene and *ADIPOQ* gene variants and environmental factors that might be associated with risk for CRC development. The study included 105 patients with CRC and 190 controls. Genotyping was performed for *RETN* rs1862513 (-420C/G) and *ADIPOQ* rs266729 (-11377C/G) single nucleotide polymorphisms (SNPs) by quantitative polymerase chain reaction. Multifactor dimensionality reduction (MDR) program followed by Permutation Testing was used to identify and evaluate potential gene-gene and gene-environmental interactions [2]. MDR analysis identified the best model consisted of *RETN*, *ADIPOQ*, exercise training, cigarettes smoking and alcohol consumption (Testing Accuracy: 0.6954, Consistency: 10/10, $p<0.001$). Statistically significant positive (synergistic) interactions were demonstrated between *RETN* and *ADIPOQ* (positive gain information of 0.15%), as well between *RETN* and cigarettes smoking (positive gain information of 0.71%). Significant epistatic interactions between *RETN* and *ADIPOQ* SNPs in combination with environmental factors may be related to risk for CRC.

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Development immunoaffinity chromatography for purification extracellular vesicles

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Cell to cell communication is pivotal for all multicellular organisms. Extracellular vesicles (EVs) are small membrane-enclosed entities released from different cell types [1]. Exosomes are the vesiclesofaproximately 30-150 nm in diameter. They are present in bodily fluids and they have been linked with both physiological and pathological conditions such as blood coagulation,immune surveillance, tumors genesis and their metastasis [2]. EVs present in bodily fluids are considered as a unique source of clinically relevant and non-invasive biomarkers. Nevertheless, the present exosome isolation approaches are very inefficient and standardized purification and characterization methods would critically improve the quality of the diagnostic information [3]. Immuno-affinity based methods for exosome purification have been shown to be effective for purification of cancer-related exosomes from blood of prostate cancer patients promoting the diagnostic values of this type of approach [4]. The main objectives of the project is to set-up a robust immune-affinity based approach for capturing exosomes from biological samples. In this work, 5 different constructs of heavy-chain-only antibodies (H1, H6, D5, G2, B1), capable of binding exosomes, were first expressed in E Coli cells.The first small scale expression test showed that the constructs were produced in soluble form. Protein were purified using metal affinity chromatography. Purity of the obtained protein preparations were confirmed by SDS PAGE.Flow cytometry was used to testthat these constructs indeed bind exosomes. The nanobody were coated onto latex beads and used to bind the EVs present in sample. Beads were incubated with EV- enriched fractions recovered from supernatant of Hek and Jurkat cells. All tested nanobodies showed increase in GFP fluorescence intensity. Then VHHwere immobilized on polimetacrilatemacroporous polymer, which then used for isolation exosomes from plasma of healthy voluntary donors. NTA (Nanoparticle Tracking Analysis) was used to determining diameter of isolated vesicles. Purification of VHH-GFP constructs by metal affinity chromatography resulted in homogenous preparations. Final yields were in range of 5 mg of protein per litre of medium. By Flow cytometry it was confirmedthat these constructs bind exosomes from two cells lines: Hek, Jurkat. Immune purification resulted in range of 50-65 µg of exosomal protein per 250 µL of undiluted plasma, and range of 210-320 µg/mL of exosomal lipids. By NTA, it was confirmed that

diameter of isolated vesicles from Hek and Jurkat cells culture, had similar profile as vesicles isolated from plasmaof voluntary donors, ranging from 100 nm to 300 nm. We have been able to set-up a cheap system for exosome capture using VHH antibodies and polymethacrylate matrices. Our approach should significantly simplify and cheapen the cost of exosome purification due to low cost of production both VHH and polymethacrylate carrier matrix enabling simplification of systematic stratification of EV sub-populations and their individual characterization.

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Quantitative detection of *Microcystis aeruginosa* in fresh water using single domain antibodies (VHHs)

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Microcystis aeruginosa in fresh water have shown to pose significant threat to aquatic bodies and human health. The toxicity of cyanobacteria metabolites urged for the development of methods for their rapid and efficient detection. What is still almost completely missing is the availability of reagents for the quantification of *M. aeruginosa* cells in water to monitor the fluctuations of its populations. Recombinant nanobodies (VHHs) can represent a source for the rapid generation of immunoreagents suitable for proper monitoring. In this study we describe how nanobodies against *M. aeruginosa* were selected in vitro from a naïve phage display library (1) through a biopaning process. Clones showing potential binding *to M. aeruginosa* were sequenced and corresponded to six unique sequences which were subcloned, purified as GFP-VHH fusions (2) and characterized for their biophysical features and finally evaluated as immunoreagents suitable for the development of a detection test based on thermal lens spectrometry (TLS). Binding specificity analysis by ELISA using purified nanobodies showed no cross reactivity with other unrelated tested algae cells whereas TLS enabled a limit-of-detection of 2.733 cells/mL.

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Chokeberry (*Aronia melanocarpa*) fruit extract modulates mouse immune response *in vivo* and *in vitro*

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Chokeberry (*Aronia melanocarpa*) is known for its strong anti-oxidant properties. Anti-inflammatory, anti-hypertensive and anti-diabetogenic activities of orally consumed chokeberry extracts have also been reported. The effects of chokeberry extract on the immune response parameters have been only sporadically assessed. Therefore, the aim of our study was to investigate the effects of orally consumed chokeberry extract on the immune response *in vivo* and *in vitro* in healthy and in diabetic C57BL/6 mice, in which diabetes was induced by multiple low doses of streptozotocin (MLDS). Chokeberry extract administered to healthy mice (50 mg/kg body weight) exerted immunomodulatory effects as evidenced by decreased proportion of $F4/80^+$ macrophages, $CD11c^+$ dendritic cells, $CD4^+$ T helper cells, $CD8^+$ T cytotoxic lymphocytes and $CD4^+CD25^-$ activated T lymphocytes within the gut-associated lymphoid tissue. Surprisingly, oral consumption of chokeberry extract in doses of either 200 mg/kg bw or 50 mg/kg bw in diabetic mice resulted in the increase of blood glucose levels. In an attempt to decipher the underlying mechanisms of chokeberry extract effects in the context of autoimmune/inflammatory disease, we have evaluated its effects *in vitro* on purified immune cells. Seemingly, the chokeberry extract exerted pro-inflammatory effects *in vitro* through the up-regulation of nitric oxide and IL-1β production in macrophages and dendritic cells, increased macrophage CD86-related activation and promotion of type 1 T helper cells ($IFN\text{-}\gamma^+$) differentiation. In addition, an increased proportion of $CD4^+$, $CD8^+$ and B lymphocytes within the spleen was observed. Collectively, the obtained results imply that our particular chokeberry extract displays pro-inflammatory characteristics and that care should be taken when chokeberry is to be included in the human diet.

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Adrenal asymmetry in expression of catecholamine synthesizing enzyme in chronically stressed rats

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The literature data confirm that the right brain hemisphere plays a dominant role in the control of both hypothalamo-pituitary-adrenal axis and autonomous functions essential for survival, while the left hemisphere predominantly affects parasympathetic functions [1,2]. In our previous studies, we found that chronic social isolation (CSI) increases catecholamine levels in the plasma [3]. The aim of this study was to examine whether there is asymmetry in sympathoadrenomedullary activity in stress condition. Therefore, in this study we investigated changes in gene expression of catecholamine biosynthetic enzymes (tyrosine hydroxylase-TH and dopamine-ß-hydroxylase-DBH) as an index for sympathoadrenomedullary activity in right and left adrenal medulla of control and chronic socially isolated rats. The investigated parameters were quantified by using real-time RT-PCR and Western blot analyses. We found that CSI produced a significant decrease in gene expression of both examined catecholamine biosynthetic enzymes in left adrenal medulla, which suggests a higher impact of sympathoadrenomedullary input in the left medulla in stress condition. Based on these results, it may be concluded that sympathoadrenomedullary activity is asymmetric. A significant decrease in gene expression of catecholamine biosynthetic enzymes in left adrenal medulla may confirm that the right brain hemisphere was more vulnerable to chronic psychosocial stress.

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Quantitation of the active alpha-2-macroglobulin by trypsin protease zymography

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Alpha-2-macroglobulin (α_2M) is a homotetrameric blood glycoprotein having molecular mass of 720 kDa which acts as a general protease inhibitor [1]. So far, the methods to estimate the quantity of α2M and its activity were separate procedures. The quantity is usually measured by immunochemical assays and the anti-protease activity of α_2M by measuring the activity of trypsin bound to α_2M using chromogenic substrate BAPNA [2]. A simple and reliable method for determination of the concentration and function of α_2M by zymography was developed. This method is based on the covalent binding of α2M and trypsin followed by non-reducing PAGE and zymography with gelatine incorporated in the electrophoretic gel. The results have shown that α_2M binds trypsin in a linear, concentration-dependent manner. The sensitivity of the method is 125 nM with an intra-assay coefficient of variation 4.2 %. Freezing of α_2M induces its partial denaturation, which can be seen as the reduction in the amount of functional molecule and its reactivity with trypsin. The method was further tested using α_2M from patients with an end-stage renal disease who are known to be under an increased oxidative stress and inflammation, which are expected to modify the structure of proteins. Using α_2M from these patients, lower affinity of α_2M towards trypsin was detected when compaired to α_2M isolated from healthy persons. The reported zymographic method enables measurement of α_2M taking into consideration both its quantity and function, stressing the importance of determination of the amount of physiologically active molecules and not just their total amount present in the sample. Monitoring of the relation quantity/activity becomes very important when the sample originates from an individual exposed to a stress or with a disease accompanied by post-translational modifications of proteins such as diabetes, renal disease or cancer [3]. Presented method also enables determination of α_2M in the presence of different modifying chemical substances.

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Metal ions as regulatory elements of artificial DNA cleaving enzymes

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Nuclease-mediated genome editing by artificial DNases offers a powerful tool for gene therapy [1]. Noteworthy, metal ions play key role both in the recognition of the specific DNA sequences in zinc-finger type domains, as well as in the catalytic action of the DNA hydrolysis in the active center. Our research focuses on new type of Zinc Finger Nucleases (ZFNs), which can be regulated in multiple manner by the help of metal ions. The C-terminal nuclease domain of the Colicin E7 protein (NColE7) contains an HNH metal binding motif at its C-terminus, which can become catalytically functional only in the spatial vicinity of the N-terminal positively charged regulatory amino acids. Regulation of the artificial metallonucleases similarly to the natural enzymes can be established by exploiting the intramolecular allosteric effect on metal ion and DNA binding, specificity, structure and catalytic activity. We have designed and synthesized various protein chimera by combining zinc fingers and NColE7 so that the N- and C-terminal regulatory and catalytic segments are separated by the zinc finger, but expected to approach each other upon specific DNA binding [2]. We elaborated a new purification method in which the affinity tag is cleaved by protease mimicking nickel(II) ions specifically targeting an SXHY amino acid sequence, while observing the additional regulatory effect of the affinity tags.

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Inhibitory effect of diclofenac to activity of catalase in *in vitro* conditions

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In this paper, the spectrophotometric method examined the influence of diclofenac on the enzyme catalase. The role of catalase is to protect cells from oxidative damage caused by reactive oxygen species, as well as from the toxic effects of hydrogen peroxide [1]. Diclofenac belongs to the group of non-steroidal anti-inflammatory drugs, and its action is based on the inhibition of cyclooxygenase activity and the consequent formation of pro-inflammatory mediators such as prostaglandins and thromboxanes [2]. Using the Lineweaver-Burk diagram, the values of the Michaelis-Menten constants (K_m) and the maximum velocity were determined without the presence of different concentrations of diclofenac. The values of the maximum velocity and the values of K_m change, which tells us that in the presence of hydrogen peroxide as a substrate, diclofenac proved to be a partial acompetitive inhibitor. In the case of partial inhibition of catalase, the decomposition of peroxide continues to be partially performed after diclofenac has been inhibited by the enzyme-substrate complex, that is, the reward complex of the enzyme-substrate-inhibitor is disorder partially on the enzyme, the product and the inhibitor.

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Fine-tuning of AMPK-ULK1-mTOR regulatory triangle is crucial for periodic activation of autophagy

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The autophagy-dependent self-eating is tightly regulated by mTOR and AMPK kinases. AMPK promotes autophagy by phosphorylating ULK1, the key inducer of autophagosome formation, meanwhile mTOR down-regulates it under nutrient rich condition[1]. However, the active ULK1 can inhibit both AMPK and mTOR [1,2]. Interestingly, a periodic activation of ULK1 was also observed during prolonged stress [3]. We claim that the negative and double negative feedback loops of AMPK-mTOR-ULK1 regulatory triangle determine an accurate dynamical characteristic of autophagic process to cellular stress (such as starvation or rapamycin-induced mTOR inhibition). In our study we suppose that a delayed negative feedback loop between AMPK and ULK1 is essential to manage a proper cellular answer upon autophagy induction. By using both molecular and theoretical biological techniques, we suggest that AMPK kinase gets induced followed by ULK1 activation during prolonged starvation or rapamycin treatment, whereas active ULK1 kinase quickly down-regulates AMPK resulting in a delayed decrease in ULK1 activity. This periodic repeat of AMPK-ULK1 activation/inactivation due to the negative feedback between them generates an oscillatory activation of autophagy, as well. We demonstrate that this periodic induction of autophagy is essential to guaranty the suitable dynamical features of the control network when mTOR is down-regulated.

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Improvement in azo dyes degradation by saturation mutagenesis of lignin peroxidase catalytic tryptophan environment

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Azo dyes are abundant environmental pollutants toxic for both plants and animals [1]. Both chemical and physical methods for their degradation or removal proven to be inefficient leaving biodegradation as the best option [2]. Lignin peroxidase (LiP) from *Phanerochaete chrysosporium*is a heme containing lignin-degrading oxidoreductase capable of peroxide-dependent oxidation of a great variety of structurally different molecules, including industrial dyes [3]. Four positions in the catalytic environment of the amino acid tryptophan those defer between ligninolytic peroxidases were mutated into all 20 amino acids and the obtained library with 10^4 independent clones was expressed on the yeast cell surface. Obtained saturation library was used for selection of variants showing elite azo dyes degradation activity and multiple cycles of dyes degradation. Elite candidates were selected, sequenced, extracted from yeast cell surface and characterized. Optimization of pH stability, redox mediator concentrations and application of preferred variants resulted in up to 15 times higher catalytic efficiency of dyes degradation for selected variants compared to wild-type enzyme.

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Responses of *Pseudomonas aeruginosa* san ai to nanoceria

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Pseudomonas aeruginosa is well known for its possibility to grow in diverse environments due to great potential for adaptation and its metabolic diversity. *Pseudomonas aeruginosa* san ai, an environmental isolate from alkaline, mineral cutting oil was previously used in various studies in our laboratory, including the study of the organism's capacity for heavy metal removal [1]. Nanoparticles in general are gaining attention for inhibition of bacterial growth including Gram negative *P. aerugunosa* [2]. Cerium oxide nanoparticles (nanoceria) act as biological mimics of enzymes involved in oxidative stress defense [3]. In this study, effects of nanoceria on *P. aerugunosa* san ai were investigated. The nanoparticles have causedsevere metabolic response of the microorganism. Production of secondary metabolites such as siderophores pyoverdine and pyochelin, and pigment pyocyanin, considred as a redox shuttle, was up regulated and accompaniend with alterations in: biofilm formation, redox homeostasis and proteome profile. Relatively low antibacterial activity of nano-CeO_2 against P. aeruginosa san ai implays an interaction of nanoparticles with molecules from outer surface of cells that efficiently protect microbial cell.

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The association between high-density lipoproteins characteristics and hepatic steatosis index

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The diversity of high-density lipoproteins (HDL) is reflected through distribution of subclasses which differ in size, content and the role in metabolism. Serum amyloid A (SAA) is an inflammatory protein that may impair HDL functionality [1]. We investigated the association between markers of HDL metabolism and hepatic steatosis index (HSI), a surrogate marker for non-alcoholic fatty liver (NAFLD), derived from anthropometric (BMI) and liver function tests (transaminases activities) parameters [2]. This study included 124 subjects classified into two groups using HSI>36.0 cutoff for determining a presence of NAFLD. HDL subclasses were separated by polyacrylamide gradient gel electrophoresis. SAA was determined by commercial ELISA. In HSI>36.0 group, we found lower relative proportion of the largest HDL 2b subclass (HDL 2b (%): HSI≤36.0-47.7±6.1, HSI>36.0-44.9±5.6, p=0.019) and higher SAA concentration (SAA (μg/L): HSI≤36.0-55.9±13.7, HSI>36.0–63.1±13.1, p=0.019). Higher SAA concentration and the redistribution of HDL subclasses may be indicative of unfavorable HDL qualitative properties in subjects elevated HSI.

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Bacillus sp. isolated from Japanese food Nattō

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Nattō is a traditional Japanese dish made from fermented soybeans and is usually combined with soy sauce. It is very rich in vitamins, amino acids, proteins, sugars, fats, minerals and dietary fibres, and polypeptides consisting of 275 amino acid residues with anticoagulant, fibrinolytic, blood pressure lowering effects and antioxidant activity [1]. *Bacillus subtilisnatto* belongs to the *Bacillus subtilis* species, and it is the basis for the production of traditional Japanese food. Enzymes and proteins of this strain also show antithrombin effects similar to heparin,as well as antitumor activity. It has also been shown that *Bacillus subtilisnatto* contains a nattokinase, which exhibits a strong fibrinolytic activity and activates other fibrinolytic enzymes [2]. The microorganism was isolated from the Japanese speciality: 1 g of Nattō was added to 9 mL of saline, resuspended and incubated in an aqueous bath at 80°C. A dilution series (10^{-1}-10^{-9}) was made from which 1 mL of culture wastaken and seeded on Petri dish with nutrient agar (peptone 1, 15 g, meat extract, 3 g, sodium chloride, 5 g, dipotassium hydrogen phosphate, 0.3 g, agar, 18 g, distilled water, 1 L) and incubated at 28 °C. Pure, individual colonies were isolated by the method of exhaustion. The isolated microorganism was characterized by API 50 CHB/E tests and 16S rRNAgene sequencing. The results of the API 50 CHB/E test showed that the resulting microorganism belongs to the species *Bacillus subtilis* with a percentage of agreement of 99.9%, with literature. This was confirmed with 16S rRNA gene sequencing.

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Molecular effects of plant extracts: Three immunochemical methods in two examples

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Many active compounds from traditionally used plants have been isolated, purified and used as supplements with broad health claims. However, the precise molecular targets of specific compounds have not always been identified. Moreover, plant extracts have been frequently proved more efficient than a single „active" compound derived from it. Of particular interest are plant extracts with potential for various health claims, such as reduction of inflammation and induction of cancer cell death. In this study, we report evidence obtained with immunochemical methods (Western blotting, immunocytochemistry and ELISA) of molecular effects produced by two plant extracts (*Vitis vinifera* and *Helichrysum italicum*) on the cells in culture. Three cell lines were used: HeLa, MCF-7 and MRC-5, and effects of extracts were recorded after 24 h and 48 h incubation periods. Pure compounds from both extracts were used as a reference. Detection of apoptosis was performed by dual acridine orange-ethidium bromide staining. Western blot analysis was done using ULK1 polyclonal antibody, while enzyme-linked immunosorbent assay NFkB p65 transcription factor was used replacing the cumbersome radioactive electrophoretic mobility shift assay. All three immunochemical methods were useful in determining specific molecular effects, which is of relevance for potential use in pharmaceutical industry.

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Modified vectors for wheat germ-based cell-free protein expression

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The wheat germ-based protein expression system has been proven to be appropriate for production of eukaryotic proteins of native conformations by a cost- and time effective-manner [1,2]. The system uses pEU3 vectors as templates of T7 RNA polymerase-based *in vitro* transcription reaction to provide the mRNA need of protein *in vitro* translation. Since the previously available GST and His_6 affinity tags [3], proved to be insufficient for labelling of recombinant proteins in certain protein-protein interaction assays, we set out to create vectors encoding further affinity tags. The modified pEU3-NII-LICNot vectors are suitable for production of proteins with: i) AviTag for protein biotinylation by BirA enzyme; ii) HALO-tag for covalent linkage of proteins to affinity purification beads; iii) His_{12}-labelling for increased detection sensitivity; iv) double tagged proteins with various motifs at the N- and C-termini; v) FLAG-tag for highly selective protein detection. The obtained results demonstrate that the novel vector constructs are suitable for synthesis of diverse recombinant proteins and enable effective purification and detection of the translated proteins. Consequently, these developments could aid the *in vitro* functional and interaction studies of eukaryotic proteins.

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Composition of gliadin proteins in bread wheat genotypes

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Gliadins are storage proteins of wheat seed endosperm which are soluble in 70% ethanol, which classified into α-, β-, γ-, and ω-gliadins with a molecular weight range of 16 to 80 kDa. The six *Gli* genes encoding gliadins are located at the short arm of chromosomes 1. and 6. on A, B and D genomes of wheat. Gliadins have impact on nutritional and technological quality of bread wheat flour. The aim of this work was study of gliadins component variability, encoding allele polymorphisms at *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2*, locus and association with dry gluten content. The 10 genotypes of wheat were included in research. At least 20 single seeds were used of each genotype for extraction of gliadins. The separation gliadin molecules conducted by using electrophoresis method at pH=3.1 on 8.33% polyacrylamide gel in electric field at the constant voltage 550 V for 3 h [1]. Electrophoregrams used for determining *Gli-1* and *Gli-2* alleles were prepared using previosuly described method [2,3]. Gluten content was determined by washing the dough with 2% saline solution, which is dried and weighed on a technical scale, the value in proportion to the initial weight of the sample represents the percentage share of dry gluten. The 4 alleles (***b, f, h, m***) at the *Gli-A1*, 5 alleles (***b, g, d, k, l***,) at the *Gli-B1*, 3 alleles (***a, b, k***) at the *Gli-D1*, 5 alleles (***a, b, e, g, k***) at the *Gli-A2*, 6 alleles (***a, b, e, o, p, v***) at the *Gli-B2* and 5 alleles (***a, b, j, k, m***) at the *Gli-D2* locus were identified. Frequency of identified alleles varied between 10% (*Gli-B1k, Gli-A2k, Gli-B2v*) and 50% (*Gli-D1b*). Content of wet gluten variate from 26.42% in G-3601-4 to 34.94% in G-3622-1 genotype which is depending on genotype. The high polymorphisms of alleles indicated genetics diversity and high frequency of alleles are results of selection in breeding programme.

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Interactions of ellagic acid metabolites with human and bovine serum albumin by fluorescence quenching of protein intrinsic fluorescence

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Urolithins are represent catabolic derivatives of ellagic acid (EA) and ellagitannins (ETs) produced by gut microbiota after consumption of different ETs [1]. Once produced these catabolites can be absorbed, circulate in plasma and accumulate in urine as glucuronide and sulphate conjugates while aglicones can be directly excreted in faeces [2,3]. The health effects attributed to urolithins are numerous and diverse, ranging from antimalarial properties to anticancer activities and regulation of gene expression. The aim of this work was to study binding of Urolithins: Urolithin-A (Uro A); Urolithin A-glucuronide (Uro AG); Urolithin-B (Uro B) and Urolithin-B-glucuronide (Uro BG); to human and bovine serum abumin by fluorescence quenching of protein intrinsic fluorescence. From the spectra obtained, the Stern-Volmer, the apparent static, and the bimolecular quenching constants were calculated. The structure of polyphenols revealed to significantly affect the binding/quenching process; in general, the binding affinity decreased with glycosylation. For Human Serum Albumin quenching constants were Uro A and B *Ksv* were 17730,54 ± 1240,71 and 17141,25 ± 2262,32 respectively, for Uro AG and BG 857,86 ± 143,05 and 1577,50 ± 225,36 respectively. For bovine serum albumin quenching constants were Uro A and B *Ksv* were 59236 ± 5706 and 69653 ± 14922 respectively, while for Uro AG and BG these values were 15179 ± 2770 and 9462 ± 1955 respectively. Higher hydrophobicity increases the binding affinity to HSA as well as BSA and could also be a reason for lower bioavailability of aglicons in sera noted in previous studies since higher rate of phenol binding to proteins is linked to reduced bioavailability.

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Suppresion of type 1 diabetes in mice by oral treatment with ATRA- and TGF-β-loaded microparticles

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Type 1 diabetes (T1D) is an autoimmune disease in which a strong inflammatory response causes the death of pancreatic β-cells. Attempts to induce anti-inflammatory/regulatory immune mechanisms that would attenuate disease progression have shown little or no beneficial effects. We introduced microparticles (MPs) loaded with Transforming Growth Factor β (TGF-β) and All-Trans Retinoic Acid (ATRA), both well-known stimulators of T regulatory cell (Treg) differentiation and stabilization. Male C57BL/6 mice were treated with multiple low doses of streptozotocin for T1D induction, and with vehicle, empty MPs, or ATRA- and TGF-β-loaded MPs for 10 days (every other day). Both T1D incidence and immune cell infiltration into the pancreatic islets was lower in ATRA/TGF-β-treated mice. In Peyer's patches (PP), ATRA/TGF-β up-regulated tolerogenic dendritic cells (tolDC). Additionally, IL-1β expression was reduced in PP, as was the ratio of iNOS/Arginase expression, reflecting a less inflammatory environment. This was accompanied by a reduced proportion of Th1 and Th17 cells and up-regulation of Treg. IL-17 expression within $CD4^+$ T cells from PP was also lower, and was accompanied by down-regulation in RORγt expression (key transcription factor of IL-17). The situation in the pancreatic lymph nodes (PLN) was similar to PP regarding the down-regulation of Th1 cells. Additionally, in response to ATRA/TGF-β treatment, the proliferation of T effector cells was reduced in PLN, while Treg proliferated more, and several crucial markers of Treg suppressive activity were increased. In conclusion, ATRA and TGF-β released from MPs successfully ameliorated T1D by potentiating tolDC and Treg responses and inhibition of Th1 cell differentiation in the draining lymph nodes, thus blocking the entrance of immune cells into the pancreatic islets and protecting β-cells from further destruction.

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The effect of dietary cadmium on *Ostrinia nubilalis* (Hbn.) larval development rate and antioxidative gene expression

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Cadmium (Cd) has a negative impact on living organisms when is accumulated in their bodies, after long term exposure, to a level that is highly toxic and strongly affect their physiological state, function and development. In this study, the effect of Cd exposure on developmental rate of *Ostrinia nubilalis* (Hbn.) larvae was examined and the gene expression of two antioxidative enzymes (*Cat* and *Gpx*) was analyzed. Newly hatched first instar (L1) larvae were placed on a Cd contaminated diet and maintained until the larvae reached their final, fifth instar (L5), or developed into a pupa. In total, four experimental groups, three treatments (concentrations of Cd in fresh diet: Cd I: 0.7, Cd II: 6.8 and Cd III: 41.7 mg/kg) and a control group (non-treated diet) were set up. Our results demonstrated that the developmental rate of *O. nubilalis* larvae was strongly reduced when were exposed to the highest concentration of Cd, 41.7 mg/kg in the fresh diet, resulting in a significantly delayed appearance of the first pupa of *O. nubilalis* that appeared 4 days later than in the control group. The expression of *Cat* and *Gpx* genes, as molecular markers of oxidative status, was significantly lower in the experimental groups Cd II and III in comparison with the control group. Long term exposure to Cd during larval development may downregulated the expression of selected antioxidative genes involved in the first line of response to heavy metal exposure.

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The effect of H_2S donor GYY4137 on T cells in experimental autoimmune encephalomyelitis

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GYY4137 is a slow-releasing donor of H_2S with anti-inflammatory properties. Experimental autoimmune encephalomyelitis (EAE) is an animal model of the human chronic demyelinating inflammatory central nervous system (CNS) disease, multiple sclerosis. The aim of the study was to examine the *in vitro* effects of GYY4137 on draining lymph node cells (DLNC) and spinal cord immune cells (SCIC) from rats with actively induced EAE. DLNC and SCIC were isolated from rats immunized with myelin oligodendrocyte glycoprotein peptide or spinal cord homogenate with CFA, respectively, and subsequently treated with GYY4137 for 24h. Having no effect on cell viability, GYY4137 reduced IFN-γ and IL-17 production in these cells. Further, it reduced percentage of IL-17$^+$ cells within the CD4$^+$ population of SCIC, but not of DLNC. Treatment with GYY4137 for 40 min, reduced the percentage of CD4$^+$CD25$^+$FoxP3$^+$ (Treg) cells in the population of DLNC, as well as in CD4$^+$ T cells purified from DLNC. Nevertheless, GYY4137 did not affect the percentage of Treg among SCIC. These results suggest that GYY4137 negatively affects regulatory T cell population at the periphery, but not within the CNS. It also reduces the ability of the encephalitogenic cells to generate the major encephalitogenic cytokines, *i.e.* IFN-γ and IL-17, both at the periphery and in the target tissue. Further studies on the relevance of the observed results for the pathogenesis and therapy of multiple sclerosis, as well as on the mechanisms behind them are warranted.

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BanLec-GFP binding to influenca virus glycans

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Many lectins inhibit the replication of viruses by interacting with viral envelope glycoproteins and prevent viral entry into cells. Banana lectin (BanLec) is mannose-specific protein which recognizes and binds to glycan structures on the surface of a wide range of viruses, including HIV, HCV, H1N1 [1-3]. Production of BanLec-GFP (green fluorescente protein) chimera could simplify further research on BanLec interactions with virus envelope glycoproteins. The aim of this study was to produce BanLec-GFP by recombinant DNA technology and to test its biological activity by binding to mannose structures on the surface of the influenza virus vaccine.The BanLec-GFP himera was produced in *E. coli*, and it was purified by combination of affinity and ion exchenge chromatography. BanLec-GFP primary structure was verified by mass spectrometry and secondary structures was analysed by CD spectrometry. Biological activity of the purified chimera was confirmed in ELISA by binding of BanLec-GFP to mannose structure on the influenza virus vaccine. ELISA inhibition was performed by binding of BanLec-GFP to influenza envelop glycans in the presence of the different concentration of BanLec wild-type and two BanLec mutants as inhibitors.

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Effects of vanadate on glutathione metabolism in mycelium of fungus *Phycomyces blakesleeanus*

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Vanadium enters mycelium of fungus *P. blakesleeanus* in both +5 and +4 oxidation states [1]. Mycelium in three stages of growth (20h, 36h and 56h) was treated with three concentrations of V^{+5} (1, 5 and 10 mM) for 1h or 5h. The decrease in viability of mycelium was noticed only at 36h for all applied concentrations. Glutathione has a role in reduction of V^{+5} to a less toxic form V^{+4} ,[2] so we assayed the changes in total glutathione. Mycelium in early exponential phase of growth (20 h), after 1h or 5h treatment, showed concentration dependent decrease in glutathione, with the largest decrease of 40±4% after 1h and 22±9% after 5h treatment induced by 10 mM V^{+5}. In stationary phase of growth (56h), changes in glutathione concentration were noticed only after 1h of treatment, and the largest decrease (36±6%) was induced by 1 mM V^{+5}. Activities of glutathione reductase (GR), glutathione transferase (GST) and glutathione peroxidase (GPx), have also been examined. Increase in the activities of GPx and GST was noticed in 56h old mycelia treated for 5h with 5 mM and 10 mM V^{+5}, but the only statistically significant increase (54±20%) was noticed in GST activity after 5h of 10 mM V^{+5} treatment.

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Antitumor effect of the chalcone analogue, (E) -1- (4-ethoxy-3-methoxyphenyl) -5- methylhex-1-en-3-one on HCT-116 cells

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Chalcones represent precursor compounds for flavonoids biosynthesis in plants. Various studies have shown that the chemical structure of chalcone is responsible for their antitumor and anti-inflammatory effect. Apart from a significant success in the treatment of variety of tumors, anti-tumor efficiency of organic heterocyclic compounds in tumor therapy is limited due to drug resistance of tumor cells, the non-selectivity of the administered drug towards healthy cells and abundance of unwanted side effects. Due to the existence of the above mentioned drawbacks, it is necessary to synthesize novel heterocyclic compounds which will have more efficient antitumor effect. The aim of our research was to investigate the antitumor effect of the (E) -1- (4-ethoxy-3-methoxyphenyl) -5- methylhex-1-en-3-one (H) on healthy MRC-5 cells (normal human fibroblast cell line) and tumor HCT-116 cells (human colon cancer cell line). Also, we compared the effect of the investigated chalcone analogue with the effect of chisplatin (cysPt), as referent substance. The results of our previous study showed that H shows a more effective cytotoxic and apoptotic effect on human cervical cancer (HeLa) cells compared to the effect of cisplatin. Concentrations of all investigated substances were 0.3; 1; 3; 10; 30; 100 μM. MTT assay was used to evaluate the effects of investigated substances on cell viability whereas flow cytometry (Annexin V-FITC/7-AAD staining) was used to detected type of cell death induced with investigated substances. The result of our investigation indicated that newly synthesized vanillin based chalcone analogues expressed powerful antitumor effect on cancer cells (HCT-116 cell line), while their effect on healthy cells (MRC-5 cell line) was not statistically significant. The result of our research shows that chalcone analogue express more powerful antitumor effect compared to the effect of referent substance. Cell death was mediated via mitochondrial apoptotic pathway.

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Plantago lanceolata L. effects gene expression of enzymes involved in prostaglandin E_2 production

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Prostaglandin E_2 (PGE_2) has numerous functions in physiological and pathophysiological conditions. PGE_2 is biosynthesized via the cyclooxygenase pathway of arachidonic acid (AA) metabolism through three cascade reactions. Firstly, phospholipase A_2 (PLA_2) release AA from membrane glycerophospholipids, then cyclooxygenases (COX-1 or COX-2) convert AA to PGG_2 and subsequently PGH_2, which is isomerized to PGE_2 by one of three PGE_2 synthases: cytosolic (cPGES) or two membrane-bound PGE_2 synthases (mPGES-1 or mPGES-2). Since PGE_2 is responsible for classical inflammation signs (pain, redness and edema), inhibition of PGE_2 production is the target of the most antiinflammatory drugs [1]. *Plantago lanceolata* L. is a plantain, highly recognized in ethnomedicine as a remedy for many pathological conditions, including inflammation. Therefore, the aim of this study was to examine its effect on gene expression related to enzymes involved in PGE_2 production. Inflammation in human U937 monocytes was induced by LPS after pretreatment with methanolic extracts of *P. lanceolata* and gene expression (PLA_2, COX-1/2, mPGES-1/2, cPGES) was measured by qPCR. *P. lanceolata* downregulated COX-2 expression by 30% and mPGES-1 expression by 12%, without a statistically significant effect on other genes. Since COX-1, cPGES and mPGES-2 are constitutively expressed and responsiable for of PGE_2 production in homeostasis, while mPGES-1 is functionally coupled with COX-2 activity (these two enzymes are key enzymes in inflammation), obtained results indicate that methanolic extracts of *P. lanceolata* contain components which could be good antiinflammatory agents with minor side effects.

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Identification of novel bile acid derivatives as 3α-HSD III inhibitors

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Members of aldo-keto reductase 1C subfamily are involved in transformation of a wide-range of endogenous and exogenous compounds, including steroids, prostaglandins and xenobiotics. They are involved in progression and developement of certain cancers, for example by steroid transformation and contribution to chemotherapy resistance. One distinctive feature of AKR1C2, also known as 3αHSD III, is that it is strongly inhibited by bile acids, and this fact might be used for specific regulation of enzyme activity. Human 3αHSD III was expressed in BL21 *E. coli* strain from a pET21 vector and purified using IMAC and SEC. Enzymatic assays were performed in 100 mM KP, pH 7.0, with phenanthrenequinone concentration corresponding to Km for 3αHSD III (0.1 mM) and excess NADPH (2 mM). Inhibitors were present at 40 uM for screening. For molecular dynamic simulations, parameters for new compounds were generated in the VMD forcefield toolkit. Compounds were docked to protein in AutoDock. Simulations were run for 1 ns in NVT ensemble for protein-cofactor-inhibitor ternary complexes. Molecular docking and enzyme activity screening both identified promising candidates for 3αHSD III inhibition. To further elucidate their potency and the structural basis of inhibition, IC50 values were examined and molecular dynamic simulations were conducted. It was shown that the potent inhibitors found so far are in the micromolar or submicromolar range, and their binding geometry in the protein molecule corresponds to that of ursodeoxycholic acid in the crystal structure of 3αHSD III molecule (PDB ID: 1IHI). Furthermore, some atoms from specific functional groups of the test compounds participate in additional interactions with the protein, contributing to the overall stability of the protein-ligand complex.

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Impact of high temperature on the accumulation of eEF1A in different cereal varieties

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High temperature stress is one of the most important environmental factors that influence cereal's growth, development and yield processes. For this reason, it is important to identify proteins involved in heat stress response of cereals and develop varieties tolerant to high temperatures.Recent studies haveshown that accumulation of eukaryotic elongation factor 1A (eEF1A) plays a role in heat tolerancein wheat.The aim of this research was to examine the impact of heat stress on accumulation of eEF1A in several cereals and to compare relative abundance of eEF1A in different cereal varieties.Flag leaves of four cereal varieties were sampled and used for research. After the isolation of proteins, immunoblot analysis was conducted foreEF1A quantification[1].The results showed differences among analyzed cereal varieties according to accumulation of eEF1A. Heat-induced accumulation of eEF1A was shown inthree investigated cereal varieties.The highest accumulationof eEF1A underheat-stress condition was found in wheat variety Anapurna. On the other hand, a decline in the relative abundance of eEF1A was shown in wheat variety Avenu under the stress condition. According to these findings, we can recommend wheat variety Anapurna for breeding new wheat with improved adaptability to high temperatures.

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Immobilized ω-transaminase ArRMut11 for the synthesis of amino-steroids

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ω-Transaminases are pyridoxal-5-phosphate dependent enzymes that catalyse the enantioselective transfer of an amino group from a donor to an amino acceptor [1,2]. They are of great interest in the industrial application since they allow for more environmentally friendly production of chiral amines, which are precursors of many drugs [2]. Increased stability of enzymes, recyclability and chemical selectivity can be achived by immobilizing an enzymes onto different support materials (polymeric resins, chitosan, MnO_2 nanorods, macrocellular silica monoliths, functionalized cellulose, etc.) [1,3]. ω-transaminase ArRMut11 (genetically engineered variant originated from *Arthrobacter sp*.) was purified, immobilized on a macroporous metacrylate based carrier and kinetically characterized. The immobilized enzyme was used for steroid biotransformation. Also, the increased stability of immobilized enzyme in comparison to the soluble one was observed.

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The dynamical characteristic of PERK targets choosing between life and death upon endoplasmic reticulum stress

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One of the most important roles of living organisms is to respond adequately in case of cellular stress events. Accumulation of misfolded proteins leads to ER stress which activates the three branches of unfolded protein response (UPR) regulated by IRE1, PERK and ATF6 proteins [1]. The main roles of UPR are to minimalize cell damages during ER stress and to maintain homeostatis by promoting autophagy-dependent self-digesting. In case of either excessive or permanent ER stress, apoptotic cell death gets induced [2,3]. Upon ER stress, PERK activates ATF4 transcription factor that has two targets, called CHOP and Gadd34 [1]. While CHOP mainly controls gene transcription involved in apoptosis, the role of Gadd34 seems to be controversory [1]. Based on our hypothesis, Gadd34 has a pivotal role in the induction of autophagy upon ER stress. This raises the question, how the two different stress response mechanisms (*i.e.* autophagy and apoptosis) could be regulated by the same PERK pathway. By using both molecular biological techniques and systems biological tools, our goal is to give a qualitative description about the dynamical behaviour of the control system by exploring the dynamical profile of the key regulatory components. We confirm a strict order between autophagy and apoptosis, regulated by PERK pathway. We detect the activation of the main elements of PERK pathway both on protein and mRNA levels during ER stress. Gadd34 gets activated even at tolerable level of ER stress, which correlates with autophagy induction and always precedes the appearance of CHOP. The protein level of CHOP shows a switch-like increase only when ER stress is non-tolerable.

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Genomic and proteomic studies of the biodegradation of 2,6-di-*tert*-butylphenol by *P. aeruginosa* san ai

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The capability of the strain *Pseudomonas aeruginosa* san ai, a environmental isolate from mineral cutting oil [1], to degrade plastic additive 2,6-di-*tert*-butylphenol (2,6-DTBP) was analyzed by genomic and proteomic methods. Collection of the genes encoding to proteins involved in catabolism of aromatic compounds, including the genes of β-ketoadipate pathway: catABC and pcaBCDG were identified in *P. aeruginosa* san ai. Genomic analysis clearly indicated a potential of *P. aeruginosa* san ai for degradation of aromatics through the *ortho*-pathway. To validate genomic data, comparative proteomics of *P. aeruginosa* san ai grown on 2,6-DTBP *versus* sunflower oil, as carbon source was employed. Total of 86 proteins were identified, of which 43 and 29 uniquely in 2,6-DTBP- and oil- amended culture, respectively. Differentially up-regulated were: muconolactone-δ-isomerase (CATC_PSEAE), a unique enzyme which contributes to aromatic *ortho* degradation *via* catechol, and acetyl-CoA-acyltransferase responsible for β-ketoadipate transformation into succinyl. CATC was not found in oil-grown culture implying its strong down-regulation. The up regulation of muconolactone delta-isomerase along with enzyme activity of catechol-1,2-dioxygenase, support the hypothesis that biodegradation of 2,6-DTPB might be passing through the *ortho*-cleavage of the ring, using β-ketoadipate pathway. Having a large potential for biotransformation of 2,6-DTBP and the strain *P. aeruginosa* san ai could be used in treatments of environment contaminated with organic pollutants.

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Anti-inflammatory activity and cytotoxicity of *Gentiana asclepiadea* L. extracts

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Gentiana asclepiadea L. (willow gentian) belongs to the Gentianaceae family and it is well known as traditional medicines for hepatitis infections, as a bitter tonic and gastric stimulant. This study aimed to analyse secondary metabolites of *G. asclepiadea* aerial parts (GAA) and root (GAR) methanol extracts, as well as to evaluate their *in vitro* anti-inflammatory and cytotoxic properties. UHPLC/DAD/(+/-)HESI-MS/MS analysis confirmed high content of secoiridoid glycosides in these extracts with gentiopicrin as a dominant compound in GAA and GCR. Besides secoiridoid glycosides, both extracts also contained different *C*-glucoflavones, while *C*-glucoxanthone, mangiferin, was present only in GAA. At a concentration of 50 µg/mL, both extracts showed a higher COX-2 inhibition activity compared to COX-1 inhibition. GAR (50 µg/mL) showed significant anti-inflammatory potential with 59.45% of COX-2 inhibition activity. The results of the COX-2 gene expression assay in THP-1 macrophages displayed that the extracts, at a concentration of 25 µg/mL, had no significant influence on COX-2 gene expression. Extracts at the doses ranging from 10 to 200 µg/mL provide good biocompatibility, without toxic effects on healthy cells (fibroblasts and keratinocytes) or human cancer cell line (HepG2). The obtained results open up the opportunity to deeply investigate promising anti-inflammatory properties of *G. asclepiadea*.

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Gender-related differences in IGF-1 concentration in patients with colorectal cancer and healthy individuals

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Previous studies have shown that insulin-like growth factor 1 (IGF1) can be used as a prognostic marker of colorectal cancer (CRC) [1]. There were only few attempts to explore the nature of complex interactions between IGF1 and serum lipids, as well as their contribution to proliferation and growth of malignant cells. Our study aimed to examine gender-related associations between serum lipids and IGF1 in CRC. IGF1 was determined by ELISA test, while total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were assessed by standard enzymatic methods. In CRC group (N=86), the male population was prevalent (f/m 26/60) while control group (N=75) comprised of 37 women and 38 men. Higher concentrations of IGF1 ($p<0.001$), TC ($p<0.001$), HDL-C ($p<0.001$) and LDL-C ($p<0.001$) were observed in control group compared to CRC. In healthy subjects, elevated IGF1 concentrations was observed in men ($p<0.01$), while women had higher levels of HDL-C ($p<0.01$). Significant correlations among IGF1 and serum lipids were recorded only in female population of healthy cohort. Positive association existed between IGF1 and HDL-C ($\rho=0.450$; $p<0.05$), while IGF1 and TC ($\rho=-0.423$; $p<0.05$), as well as IGF1 and LDL-C ($\rho=-0.450$; $p<0.05$) negatively correlated. Regarding CRC group, only IGF1 showed differences across genders, with higher values in males ($p<0.01$). Our results suggested that IGF1 might have a protective role in healthy women favorably influencing lipid metabolism, while this association was not registered in CRC.

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Prophylactic treatment by recombinant banana lectin affects innate immune response in TNBS induced colitis

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A prophylactic impact of recombinant banana lectin (rBanLec), a manosa-specific lectin exerting immunomodulatory activity [1,2], was investigated in a murine model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. Particular focus was on changes in parameters of oxidative stress due to rBanLec treatment. Experimental colitis was induced in C57BL/6 mice by an intrarectal application of TNBS/50% EtOH. rBanLec treatment (0.1 μg/ml (rBL0.1), 1 μg/ml (rBL1) and 10 μg/ml (rBL10)) was performed 24h prior to induction of colitis. The impact of rBanLec treatment was assessed at the peak of the disease. Group of mice treated by TNBS/50% EtOH without rBanLec treatment (PC) was referent. A significant reduction in disease severity (evaluated according weight loss) was noticed in rBL0.1 group and it correlated with diminished infiltration of leukocyte in the colon. Inflammation-related parameters (MPO activity, production of NO, IL-12 and TNFα) were significantly lower in colonic samples taken from rBL0.1 group compared to rBL1 and rBL10 groups as well as PC group. Furthermore, the levels of regulatory cytokines (IL-10 and TGFβ) were the highest in rBL0.1 group comparing to other rBanLec-treated groups. Activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione transferase) were significantly increased in rBanLec-pretreated groups, especially in samples taken from rBL0.1 group. Presented results show that application of rBanLec in low dose prior to the induction of colitis has a positive effect i.e. it reduces the severity of experimental colitis. Observed positive impact is based on the activation of local regulatory and antioxidant mechanisms that reduce proinflammatory response in acute colitis induced by TNBS.

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Ki-67 proliferation index and expression of vimentin in three different continuous cell lines

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Ki-67 is a nuclear protein which is necessary for cell cycle proliferation, while vimentin is often used as a marker of mesenchymally-derived cells [1,2]. Expression of these markers can contribute to better interpretation of tumour cell proliferation and cytoarchitecture during both normal development and metastatic progression. The objective was to Determine Ki-67 proliferation index and vimentin expression by means of indirect immunofluorescence in BHK-21/C13, MCF-7 and MRC-5 continuous cell lines. The research was conducted on continuous cell lines BHK-21/C13, MCF-7 and MRC-5. The cell lines were held in incubator on 37 ºC, in atmosphere with 100% humidity and 5% CO_2 during the 48h. The cells were subjected to immunofluorescence for Ki-67 and vimentin antigen. Afterwards, every cell line was photographed and the photos were processed in *Fiji s*oftware. Ki-67 proliferation index and the immunofluorescence intensity of vimentin antibody have been determined. Positive expression of Ki-67 antigen was noticed in all three cell lines. Proliferation index in BHK-21/C13 was 97.01%; in MCF-7 was 90.43%; in MCR-5 was 90.58%. Vimentin intermediate filament was present in the cytoplasm of all examined cell lines. Highest values of immunofluorescence intesity for vimentin antibody were shown in BHK-21/C13, while the lowest values were shown in MCF-7. Ki-67 antigen represents a good proliferation indicator while vimentin refers well to cytoarchitecture of examined cell lines.

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Glycosylation of the human serum transferrin as a biomarker of healthy ageing

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Aging is associated with significant changes in metabolism affecting almost all physiological systems and resulting in gradual alteration (reduction) of functions. Regulatory mechanisms involved in (healthy) aging and lifespan are complex and remain unclear. Many age-related changes have been characterized, but their influence on health is insufficiently understood. Specific condition, such as ageing, may cause alteration in glycosylation pattern of serum proteins. In this study, the potential of aberrant transferrin (Tf) glycosylation as a clinical biomarker for aging was investigated. Lectin-based microarray is a simple, high throughput and very sensitive technique that enables direct glycoprofiling of N- and O-glycans, both in pure and crude glycoprotein samples. The release and purification of glycan moieties is avoided (fragmentation of protein or glycan separation), enabling comparative analysis and differential glycoproteomics. Human serum samples (n = 80) were collected from healthy volunteers: middle-aged (40-59 years old), old-aged (60-80 years old) and very old-aged (>80 years old). By using lectin-based microarray, significant differences in Tf glycoprofile between middle-aged population and old-aged people were found. Six lectins, out of fourteen tested, heve shown distinct age-associated changes (AAL, ConA, PHA-E, RCA, SNA and WGA). Tf from healthy old people has an increased level of fucosylation and glycan branching compared to the middle-aged persons. Pronounced differences were seen between 60-80and >80 years old groups, and in some cases between males and females. Altered glycosylation of Tf, thus, can be taken into consideration as a potential biomarker of physiological changes which occur due to ageing.

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Kinetics of amyloid fibril formation in the presence of metal ions and low-molecular-weight compounds

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Proteins are prone to structural changes due to their marginal stability. There are multiple pathways of structural rearrangements leading to misfolding and aggregation among which amyloids stand out as highly ordered and remarkably stable forms which appear to be a global minimum of protein free energy landscape of all proteins. *In vitro* studies on different proteins show that destabilizing conditions that favor the state of molten globule are likely to lead to ordered fibril formation. The presence of various organic and inorganic molecules was reported to affect amyloid fibril formation, eighter as stimulators or inhibitors. We investigated the formation of amyloid fibrils of human serum albumin, ovalbumin and papain in the presence of metal ions, as well as low-molecular-weight compounds. Proteins were incubated in destabilizing conditions optimized to prolong the solubility of molten globule state and induce amyloid-like structural changes. The effects of inorganic and organic additives on fibrillation process were monitored using Thioflavin T fluorescence, 8-Anilinonaphthalene-1-sulfonic acid fluorescence, Attenuated total reflection Fourier-transform infrared spectroscopy, electrophoretic and microscopy techniques. Our results show that the kinetics of amyloid formation is dependent on the presence of iron, copper, zinc and aluminum salts, as well as different lipophilic low-molecular-weight compounds. While some compounds act as complete inhibitors of fibrillation, others increase the rate of fibrillation process and promote the formation of mature fibrils.

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Influence of dose size on the metabolism of methyl and isopropyl *N*-methylanthranilates

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Recently, we investigated organ- and urinary-metabolite profiles of two polypharmacologically active Mexican orange (*Choisya ternata* Kunth (Rutaceae)) essential-oil constituents, methyl (MMA) and isopropyl *N*-methylanthranilates (IMA) [1]. Herein, we report on the influence of dose size (2 and 5 g kg^{-1}, *i.p.*) on their metabolism in rats. GC-MS analysis of the Et_2O extracts of selected tissues homogenates (liver, kidneys, heart, brain, lungs, quadriceps femoris muscle, and spleen) of rats treated with MMA and IMA enabled identification of 12 and 16 anthranilate-related compounds, respectively. The principal metabolites of MMA were the products of ester hydrolysis, *N*-methylanthranilic (NMAA) and anthranilic acids (AA), while the unmetabolized IMA and NMAA were the most abundant anthranilate-related compounds in the tissues of IMA-treated rats[1]. For both *N*-methylanthranilic acid esters, regardless of the administered dose, the chemical composition of the extracts was qualitatively very similar, but there were several differences in the relative abundances of some metabolites. In the case of MMA, the major difference was that the 2.5-times higher dose of MMA resulted in ca. 10 times higher amount of MMA and NMAA in the brain tissue. For IMA, the major difference was that at the dose of 5 g kg^{-1} a high amount of the unmetabolized compound was detected in the spleen tissue in comparison to its content in other organs, whereas at the dose of 2 g kg^{-1} the spleen contained only traces of unmetabolized IMA. Another peculiarity is that the relative ratio of NMAA and IMA in the liver and kidneys of IMA-treated rats, at the dose 5 g kg^{-1}, was significantly higher than that detected at 2 g kg^{-1}.

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Proapoptotic activity of *Gentiana punctata* L. on colorectal cancer cells

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The plants from family Gentianaceae, including the *Gentiana punctata* L., show different pharmaceutical activities and deserve attention for medicinal application regards to their chemical contents [1]. This study provides data about proapoptotic activity of *G. punctata* methanolic extract regarding to its phenolic content and redox potential in HCT-116 and SW480 colorectal cancer cells. The phenolic content in the extract is 48.64±0.55 mg GA/g while concentration of flavonoids is 21.49±0.56 mg RuE/g. The extract showed proapoptotic activity on both cell lines, induced changes in cellular morphology typical for apoptotic cells stained with acridine/orange ethidium bromide method[2], while the necrosis was not observed. The percentages of induced apoptosis were ranged between 4.53 to 17.56% in HCT-116 cells and 15.60 to 22.85% in SW480 cells, depending of concentration and time of exposure, observed on 24 and 72 h. The apoptosis was induced due to increased protein expression of Fas receptors on both tested cell lines, increased activity of caspase 8 and 9 in HCT-116 treated cells compared to control, while changes in caspases activity in SW480 was not significant. The treatments by *G. punctata* extract caused change in redox status in colorectal cancer cells. Mainly extract induces prooxidant activity by increasing $O_2^{\cdot-}$ concentrations on 24 h in both cell lines. Other parametrs indicate antioxidant properties by reduced iNOS protein expression and NO level, as well as enhanced antioxidant protection by increasing of GSH level in treated cells. Based on observed results, ability of *G. punctata* to affect apoptotic signal molecules in cancer cells and modulate redox status can be used for design of drugs originating from nature, with desirable properties in prevention and treatment of cancer.

Acknowledgements

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Epigenetic enzyme screening assay with *in vitro* reconstituted human chromatin

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Epigenetics has become an outstanding discipline since its role was demonstrated in human health, aging, tumors, and chromatin modifying enzymes became a potential drug target. Epigenetic pathways are sophisticated and large amounts of data are accumulated, but a method to produce unmodified chromatin substrates for enzyme assays is still missing. Our aim is to set up a novel, straightforward *in vitro* chromatin assembly method and histone modifying enzyme assay that serves as a universal investigation tool to test epigenetic modifications. We established a protocol for *in vitro* reconstitution of chromatin with *D. melanogaster* histones by using cell-free translation[1] and we proved that translated histones lack post-translational modifications. In our current project, we clone human core histones (histone H2A, H2B, H3, H4) and a panel of human histone modifying enzymes: writers (methyltransferases, acyltransferases, kinases, ubiquitin ligases) and erasers (demethylases, deacetylases, phosphatases, deubiquitinase). Using unmodified, reconstituted human chromatin produced by wheat germ-based, *in vitro* translation method we perform enzyme assays with the modifying enzymes and analyse our samples with the state-of-art mass spectrometry (Q Exactive plus Orbitrap). We aim to demonstrate the proof of concept by confirming known histone modifications and determine unknown ones. As a promising preliminary result, we succeeded to modify *in vitro* assembled chromatin and identify histone modifications. Ultimately, our method can be a tool to study the chromatin of different organisms, to test histone variants, to gain *in vitro* data for complementing *in vivo* observations and it can greatly contribute for a deeper understanding of epigenetic processes.

Acknowledgements

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Employment of mouse-derived intestinal 2D organoids for evaluation of major kiwi-fruit allergen Act d1 effect on intestinal epithelial cells

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Actinidin (EC 3.4.22.14) is an abundant papain-like cysteine protease present in kiwifruit, and a major kiwi fruit allergen denoted as Act d 1. It preserves its proteolytic activity and immunological reactivity under simulated gastric digestion[1]. Act d 1 exerts direct proteolytic cleavage of occludin and leads to disruption of epithelial barrier function[2-4]. Mechanisms underlying the induction of the immune response to Act d 1 and its mode of action on the gastrointestinal barrier are not fully clarified. Therefore, the aim of this research was to evaluate the effect of Act d 1 in mouse-derived intestinal organoids employed as an *ex vivo* model system. Effects of Act d 1 were analyzed in terms of structural changes and gene expression of tight junction (TJ) proteins, as well as up-regulation of pro-inflammatory cytokines (IL-1β, TNFα) and epithelium-specific IL-33. 2D intestinal mice organoids were treated with active or inactive Act d 1. Evaluation of the treatment's effect was analyzed at the genetic level by qRT-PCR as well as on the protein level by ELISA. Effect of biologically active Act d 1 on the TJ network was evaluated by immunostaining of the 2D intestinal mice organoids. In response to the Act d 1, 2D intestinal organoids produce pro-inflammatory cytokines. In addition, Act d 1 led to upregulation of TJ proteins (ZO-1, CLDN1-4, OCLDN) and pro-inflammatory IL-33, with an exception of E-cadherin, where a downregulation was observed. Act d 1 induced the disruption of the TJ network (ZO-1, CLDN-3 and E-cadherin) of mice 2D organoids through its protease activity. Proteolytically active Act d 1 was able to induce the innate immune response in mice-derived intestinal 2D organoids through the release of Th2 related cytokines, and affects tight-junction proteins on both the genetic and protein level. This finding contributes to the clarification of the molecular mechanisms in development of kiwifruit allergy.

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Comparative analysis of DNA extraction methods from human buccal swabs and fish tissue samples

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Genom is the most studied model system in molecular biology and genetic research. There are many used DNA extraction methods which vary in processing time, yield and purity, cost, ease of use [1]. The aim of the study is to isolate the total DNA from selected human and fish samples and establish a protocol that gives the best yield and proper purity of DNA material. For extraction, samples of buccal mucosa are taken from ten human volunteers and tail fin of two *Thymallus thymallus* L. specimens. In protocol 1, DNA extraction was performed with a method by Aidar [2], protocol 2 by salting method and protocol 3 with the GeneJET Genomic DNA Purification Kit. Concentration and purity of DNA were measured at Eppendorf BioPhotometer. Results for human samples showed that Purification Kit gives the highest concentration of extracted DNA but the worst purity. This method has better yield because extraction is performed in colon tubes but need further purification. In contrast, a method by Aidar gave the best purity which may suggest that isopropanol has an important role in DNA purification. By analyzing fish samples, both methods gave good yield and purity. The results implicate that DNA yield from the tissue is much higher than from buccal mucosa cells. In conclusion, it is recommended to use the Purification Kit for constant and high DNA yield but consider further purification for both human and fish DNA.

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Fructose consumption affects glucocorticoid receptor signaling and increases lipogenesis in the liver of young female rats

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The effects of early-life fructose consumption and their relation to metabolic diseases risk in adulthood are not yet elucidated. This study explored the direct effects of a diet regime characterized by fructose enrichment on glucocorticoid receptor signaling in the liver of female rats immediately after weaning. 21 day-old female Wistar rats were subjected to a 9 week-long diet regime involving standard chow in combination with either the 10% fructose solution or tap water. Glucocorticoid receptor hormone binding parameters, intracellular distribution of this molecule as well as the expression of its target genes involved in lipid metabolism (most notably Lipin-1) and glucose metabolism (PEPCK), were measured. An increase in the hepatic glucocorticoid receptor hormone binding activity as well as an elevated nuclear translocation of the receptor, in concert with the increased protein levels of Lipin-1 were observed after fructose enriched diet. This was preceeded by a hepatic elevation in Glut-2 fructose transporter expression. Fructose-enriched diet starting immediately after weaning enhanced hepatic glucocorticoid signaling in young female rats and promoted lypogenesis as evidenced not only by the lipin-1 increase but also by FAS, ACC and SCREBP-1 expression elevations contributing to hypertriglyceridemia and the expansion of the visceral adipose tissue [1], with no effect on the hepatic gluconeogenesis. These results imply that while most parameters remained within physiological reactivity, prolonged treatment might ultimately lead to more pronounced metabolic disturbances.

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Association of fatty liver index with obstructive sleep apnea

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Obstructive sleep apnea (OSA) is associated with metabolic and cardiovascular disorders, including metabolic syndrome, dyslipidemia, insulin resistance and hypertension.[1] Development of non-alcoholic fatty liver disease as important component of metabolic syndrome may be stimulated by OSA.[1] The aim of this study was to determine whether degree of OSA is associated with fatty liver index (FLI).Total of 169 examinees underwent polysomnography for determination of presence and degree of OSA. Anthropometric and biochemistry markers were obtained for all participants. FLI was calculated by online calculator using body mass index, waist circumference, gamma glutamyltransferase and triglycerides. Participants with severe OSA [median (interquartile range) - 25.5 (8.5-41.0)] had the highest FLI compared to mild-to-moderate OSA [4.0 (2.0-6.0)] and control group 2.0 (0.0-5.0)] (p=0.002). FLI correlated significantly positively with apnea-hypopnea index (AHI), C-reactive protein and glucose, but negatively with high density lipoprotein cholesterol. Multivariate binary logistic regression analysis demonstrated independent positive association between FLI and AHI (odds ratio 1.041 and 95% confidance interval: 1.007-1.076; p=0.017). Degree of OSA could be an independent determinant of FLI.

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Functionalized nanobodies: A bio-recognition molecule for the detection of the toxic microalgae *Alexandrium minutum* by means of an electrochemical immunosensor

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An immunosensor based on self-assembled monolayer of L-cysteine coupled with gold nanoparticles modified glassy carbon electrode was developed for the detection of toxic microalgae *Alexandrium minutum.* The modified electrode was prepared by immobilizing onto the surface the Spy-tagged functionalized nanobody C1 by site-specific covalent attachment through SpyCatcher. Electrochemical Impedance Spectroscopy and Cyclic Voltammetry analyses were used for characterization of the stepwise assembly and of interfacial properties of the immunosensor as well as for *A. minutum* AL9T cell quantification. A linear relationship of charge transfer resistance and cell concentration was obtained in the range of 10^3 to 10^9 cells L^{-1} with a detection limit of 5×10^3 cells L^{-1}. The potential practical application of the immunosensor was successfully assessed by quantifying AL9T cell concentrations in spiked seawater and brackish water with recovery rates between 82.1% and 113.4%. The immunosensor exhibited good reproducibility, specificity and selectivity making it a potential tool for monitoring and early detection of toxic microalgae in seawater.

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Bacterial surface display of nanobodies against cancer and toxic micoalgal cells

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The production and the outwards display of antibodies on biosensor surfaces are time consuming and expensive steps. Antibody fragments (nanobodies) suitable for antigen immunocapturing enable alternative approaches. Nanobodies were displayed on the surface of *Escherichia coli* through autotransporter secretion mechanism and such bacteria were directly coated on surfaces to serve as immunoreagents for detection of cancer cells and toxic microalgae. The nanobody-displaying bacteria were also genetically engineered for the coexpression of green fluoresence protein in their cytoplasm and used as fluorescent immunoreagents for the quantification of relative affinity to the target cell by flow cytometry analysis and surface display ELISA. For efficient functionality of the system, we optimize the bacterial adhesion on solid surfaces by optimizing the functionalization strategies and the washing steps. Our study showed the feasibility of the approach for inexpensive diagnostic applications without the necessity to purify the nanobodies.

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Serum transferrin glycopattern in patients with an end-stage renal disease: a lectin-based protein microarray

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Glycosylation pattern alterations of serum transferrin (hTf) already serve as a clinical biomarker (congenital glycosylation disorders, long-term alcohol abuse). Peritoneal dialysis (PD) is a life-saving treatment for patients with an end-stage renal disease (ESRD). When treating ESRD, peritoneum is used as a membrane for transfer of waste products from the circulation to a glucose-based medium. Prolonged and frequent exposure of peritoneum to such solution can cause peritonitis, fibrosis and inflammation, disabling further PD treatment. ESRD and PD were shown to be related to changes in the N-glycosylation pattern of serum proteins. In this study, hTf glycosylation was studied. By using a microscale purification protocol for the isolation of hTf and lectin-based microarray for glycan analysis, significant differences in hTf glycoprofile between healthy population and ESRD/PD patients were found. Out of 16 tested lectins, 8 strongly interacted with hTf. Regression analysis of the obtained results indicated statistically significant differences between two groups of samples for 5 lectins. Significant differences between groups were observed in the case of 9 lectins when specific lectin-lectin ratios were calculated. Lectin-based microarray is a simple, sensitive and high-throughput technique that enables analysis of N-glycans without fragmentation of a protein or separation of glycans, and this lectin-based microarray platform developed for differential glyco-analysis of hTf represents a good starting point for the establishment of a novel functional biomarker in patients with ERSD/PD and associated complications.

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Amplification of aptamer libraries using a modified nucleotide

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Aptamers are single stranded DNA or RNA oligonucleotides that have similar affinity and selectivity to that of antibodies; therefore, they are suitable for diagnostic and therapeutic puposes. These molecules are in vitro selected from a randomized library and can be labelled on demand. So far, only a tiny fraction of selected aptamers made their way into therapeutics due to many factors including their short half-life *in vivo* [1]. This can be salvaged by introducing non-natural nucleotides into the aptamer library. The modified nucleotides can also introduce new physico-chemical properties to aptamers, *e.g.* hydrophobic side chains can be added [2]. Here we demonstrate a method on the generation of an aptamer library that contains a tryptamino-uracil analogue (TAdUTP) instead of thymine. In our work, we studied the incorporation of the modified nucleotide by several enzymes in PCR including Q5U, Vent(exo-) and PWO polymerases. To determine if TAdUTP was successfully inserted during PCR, the PCR products were sequenced. Using the most promising enzyme, aptamer libraries were successfully amplified using emulsion PCR to minimize by-product formation. Our protocol yielded an aptamer library bearing tryptophane-like side chains that can further expand the possibilities of aptamer-target interactions and are also expected to increase their *in vivo* durability.

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Analyzing biological and chemical properties of Turkish and instant coffees

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Coffee is the third most consumed beverage in the world. Apart from caffeine, coffee is a rich source of dietary antioxindants, vitamins, fiber, macro- and microelements, alkaloids, as well as phenolic compounds, best known for their antioxidant, anti-inflammatory, anticancer, cardio- and neuroprotective properties. Different processing of the beans and preparations of the drink could affect the chemical properties and bioactivity of coffee [1]. Thus, the aim of this study was to compare the antioxidant properties of Turkish and instant coffee from different manufacturers, conducting spectrophotometric assays that include scavenging of diphenylpicrylhydrazyl and hydroxyl radicals, and Ferric Reducing Antioxidant Power assay, as well as determining total phenolic, flavonoid and tannin contents [2]. In all three antioxidant assays there was no clear distinction between Turkish and instant coffee, and no differences were noticed between brands, with an exception of hydroxyl radicals scavenging, where there were greater variations within instant coffee brands. As for total compound content, higher amounts of phenolics and tannins were present in Turkish coffee compared to instant coffee, and the regression analysis pointed out a direct correlation between the activity and phenolic and tannin content of the extracts. The results from this study suggest that both Turkish and instant coffee are a good source of antioxidants and that the processing and preparation of coffee affect the phenolic composition rather than the antioxidant activity.

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Comparing antioxidant and chemical properties of black and green tea

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Tea is produced from the leaves of *Camellia sinensis* and according to the degree of fermentation, it can be classified as black (fermented) and green (unfermented) tea. Health benefits that are linked to regular tea consumption, such as reduced risk of cardiovascular, neurodegenerative and liver diseases, anti-inflammatory, anti-diabetic activity and weight loss are mostly attributed to the presence of catechins, compounds with good antioxidant properties that belong to the class of polyphenols [1]. Since leaves are processed differently, the aim of this study was to evaluate its affect on the antioxidant activity of black and green tea of various manufacturers, through scavenging of diphenylpicrylhydrazyl (DPPH) and hydroxyl radicals ($HO^{\bullet}$), and Ferric Reducing Antioxidant Power assay (FRAP), as well as to compare total phenolic, flavonoid and tannin contents [2]. A clear distinction between black and green tea is evident in the $DPPH^{\bullet}$ scavenging and FRAP assays, as green teas expressed a better activity and also a slightly higher phenolic and tannin content. Scavenging of $HO^{\bullet}$ was in a direct correlation with total flavonoid content according to the regression analysis and all investigated teas had a similar activity that did not depend on class or brand. Presented results suggest that fermentation process does affect the antioxidant activity as green unfermented tea yielded better results, while the chemical composition was also moderately affected.

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Isolation of anti-extra-cellular vesicle single-domain antibodies

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Over the past few decades, prokaryotic and eukaryotic cells have been found to release vesicles that play a key role in cellular signaling. Extracellular vesicles (EVs) are found in a large number of body fluids, including blood, urine and cerebrospinal fluid. They have a major role in a large number of processes in our organism, starting with coagulation, immune suppression, inflammation, tumor growth and metastasis [1]. EVs specifically deliver their cargos thanks to surface displayed proteins that have affinity for target-cell receptors. Their stable lipid bilayer forms a relatively large internal volume in which regulatory messengers (nucleic acids, lipids, proteins, and metabolites) are protected during transport and finally released by internalization or direct fusion with target cell membranes. Since EVs are easily accessible in biological fluids, they are evaluated as diagnostic and prognostic biomarkers in liquid biopsy assays [2,3]. The aim of this work was to purify and characterize single-chain antibodies specific for EVs. Nanoantibodies were obtained by selecting a naive library of VHH antibodies from llama directly on exosomes originating from two different cell lines: the human embryonic kidney cell (HEK 293) and the HER2 positive human breast adenocarcinoma cell (SKBR3). After selecting the nanoantibodies, they were subcloned into a modified pET14b vector so as to obtain a construct consisting of eGFP and 6 × His tag at the C-terminus. Nanoantibodies were expressed in *E.coli* SOX strain, and purified on metal-affinity chromatography enabled by His tag at the C terminal of the construct. Flow cytometry has proven that nanoantibodies recognize specific surface molecules of exosomes. Isolated nanoantibodies were able to immobilize EVs from both cell culture supernatant and biological samples (human plasma), to be used in flow-citometry and immune-purification. A qualitative analysis of the exosomal purification using recombinant antibodies H1 and H6 was done by electron microscopy. Electron microscopy has shown that isolated EVs (50-200 nm) fall within the range defined of extracellular vesicles. In this work we report the first case of successful isolation of anti-EV nanoantibodies for the use immunoaffinity-based EV capture by direct panning of a phage library on partially purified EVs. This achievement provides stable

immunoaffinity-based EV capture and consequently simplifies the future discovery of novel antibody-vesicle surface biomarker pairs.

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Chronic restraint stress changes catecholaminergic turnover in rat hippocampus

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Chronic restraint stress (CRS) provokes anxiety and depressive-like behaviours in rats [1]. In our previous studies, we found that CRS induced significantly decreased concentrations of dopamine (DA), but increased concentrations of noradrenaline (NA) in the rat hippocampus [1], which confirmed that the hippocampus was particularly sensitive to chronic stress [2]. Also, our earlier research confirmed that CRS decreased gene expression of tyrosine hydroxylase, a "rate-limiting" enzyme of catecholamine (CA) biosynthesis, which probably confirms the decrease of *de novo* synthesis of CA [3]. However, very little is known about hippocampal turnover of CA (DA and NA) in animals exposed to CRS. Because of the direct involvement of catecholaminergic signaling in modulation of brain functions in stress condition detection of regulatory molecular mechanisms by which CRS changes catecholaminergic turnover in the hippocampus may by very important in the research of numerous diseases caused by chronic stress. Therefore, in this study we investigated how CRS (2 h × 14 days) affects the gene expression of key enzymes involved in the conversion of DA into NA (dopamine-β-hydroxylase-DBH), reuptake (dopamine transporter-DAT) and storage (vesicular monoamine transporters-VMATs) of CA in the rat hippocampus. The investigated parameters were quantified by real-time RT-PCR and Western blot analyses. We found that CRS increased gene expression of DBH, DAT, VMAT2, in the hippocampus. These results indicate: the increase of uptake of DA via DAT, the increase of storage of CA and the increase of conversion of neurotransmitter DA to NA, which is followed by increased noradrenergic capacity in the hippocampus in conditions provoked by CRS. Increased noradrenergic capacity in the hippocampus is important adaptive phenomenon of the catecholaminergic system in chronically stressed rats. Our findings indicate that CRS may be a good animal model in research of catecholaminergic turnover in rat hippocampus in stress condition.

Acknowledgments

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Immunohistochemical assesment of folate and B_{12} inhibiting non-oncologic drugs influence on cancer

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Purpose was to investigate the effect of metformin, caffeine, itraconazole and nitroglycerin, which are established non-oncologic drugs [1-2], on an *in vivo* solid tumor model of fibrosarcoma in hamsters. Thirty Syrian golden hamsters of both sexes with the approximate body weight of 100g were randomly distributed in 4 experimental and 1 control groups, with 6 animals in each group. BHK-21/C13 cells were injected subcutaneously into the back of each animal in 5 groups. The experimental groups were treated with metformin, caffeine, itraconazole and nitroglycerin via a gastric tube on a daily basis, immediately after tumor inoculation. Ki-67-positivity and cytoplasmic marker (CD34, COX4, GLUT1, iNOS) immunoexpression in the tumor samples were quantified. Metformin, caffeine, itraconazole and nitroglycerin diminished tumor mitosis ($p < 0.05$), vasculature ($p < 0.05$) tissue penetration, and increased necroses in tumor slices. Folate and B_{12} inhibiting nontoxic drugs, might be effective non-toxic agents in anticancer adjuvant and relapse prevention therapy.

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The anticancer effect of folate, B_{12} and glucose metabolism inhibiting non-oncologic drugs on animal model

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Objective was to investigate anticancer effects of metformin [1,2], caffeine, nitroglycerin and their combinations on fibrosarcoma in hamsters. The 42 Syrian golden hamsters (~100 g, both sexes), were randomly allocated in 6 experimental and 1 control groups (6 animals in each). After subcutaneous inoculation of BHK-21/C13 cells to all animals, the experimental groups started peroral daily treatment with metformin, caffeine, nitroglycerin and their combinations via gastric probe. After 2 weeks, all animals were sacrificed, blood and main organs analysed, tumors excised, weighed, diameters and volume measured. Tumor samples were pathohistologically and immunohistochemically (Ki-67, CD 31, COX IV, GLUT-1, iNOS) assessed. Ki-67-positive cells in the tumor samples were quantified. Only combination of metformin (500 mg/kg) with caffeine (100 mg/kg) and combination of metformin (500 mg/kg) with nitrogycerin (50 mg/kg) significantly inhibited fibrosarcoma growth in hamsters without toxicity. Administration of metformin with caffeine or nitrogycerin might be an effective and safe approach in novel nontoxic adjuvant anticancer treatment.

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PON1 plasma activities in aging after fish oil supplementation

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Serum paraoxonase (aryldialkylphosphatase), is an esterase protein synthesized by the liver and released into the serum, where it is associated with HDL lipoproteins. PON's might confer protection against coronary artery disease by hydrolyzing oxidized phospholipids in LDL and HDL particles as well as against homocysteinylation, two important early steps in the pathogenesis of atherosclerosis. The cellular and subcellular localization of PON1 in different tissues is of interest because it could provide additional information about its physiological role. Our aim of study was to examine PON1 activities in experimental model of aging after fish oil supplementation. Experiment were performed on young Wistar rats-3 months, (n=10, 283±7.3g) and aged Wistar rats-18months, (n=10, 380±6.3g) while plasma paraoxonase activities (U/L), (Sciavon,1996) were measured spectrofotometricaly. (Cecil Ce 2021 UV/VIS, 405nm). Treatment lasted 6 months, daily dose of fish oil was 200μL (45mg EPA, 30mg DHA). Results showed that treatment in both young and aged rats increased PON1 activity. In young rats with high significance (141.10±3.91 vs 152.67±4.80) ($p<0.001$) while in aged (115.16±9.04 vs 126.33± 6.74) ($p<0.05$). Fish oil six weeks treatment in aging seems to increase activities of PON1 in plasma which might confer protection against coronary artery diseases and atherosclerosis despite this effect was more pronounced in young Wistar rats.

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Modulation of peritonal murine macrophages functional characteristics by Bet v 1-BanLec chimera

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Allergen-specific immunotherapy (ASIT) is suitable treatment for patients with respiratory allergies. Banana Lectin (BanLec) is mannose-specific protein and can be used to deliver particular therapeutic to target cells since they are decorated by different glycan structures [1]. BanLec binds murine peritoneal macrophages and modulates their functional characteristics by binding to oligosaccharide structures on TLR2 [2]. This makes BanLec attractive protein for ASIT. Combining purified allergens with different vector systems or adjuvants can improve their immunogenicity [3]. The aim of this study was to test immunomodulatory potential of Bet v 1-BanLec chimera on murine macrophages in terms of upregulation and secretion of cytokines. The recombinant construct of the major birch (*Betula verrucosa)* pollen allergen Bet v 1 and BanLec has been designed and produced by recombinant DNA technology. Biological activity of the purified construct was confirmed by binding of Bet v 1-BanLec to a horseradish peroxidase glycoprotein in ELISA. After incubation murine peritoneal macrophages to different concentrations of Bet v 1-BanLec we demonstrated its immunomodulatory potential by measuring cytokine level, and level of expressed genes. Binding of FITC labelled Bet v 1-BanLec chimera to surface glucoproteins of murine macrophages was confirmed by confocal microscopy. Furhted *in vitro* studies will evaluate immunomodulatory potential of BanLec-Bet v 1 for application in ASIT.

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The capability of different TGF-β isoforms to induce EMT in human conjunctival epithelial cells

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Conjunctival fibrosis often emerges after infections, inflammations and mechanical stresses of the eye and when severe results in impaired vision. Recent data documented unavoidable role of epithelial to mesenchymal transition (EMT) in every fibrotic process including fibrosis-based eye conditions. The aim of this work was to establish whether human conjunctival epithelial (HCjE) cells are prone to EMT induction after prolonged treatment with well-known EMT inducers TGF-β proteins, and to test capabilities of TGF-β1, TGF-β2 and their combination to trigger this process. While TGF-β2 induced only alterations in cell-cell adhesion, TGF-β1 and combination of TGF-β proteins induced prominent change in cell morphology reflected in loss of cell-cell contacts, changes in shape from epithelial polygonal to spindle-like shape typical for mesenchymal phenotype and acquired ability to move. Statistically significant reduction of mRNA expression of epithelial marker genes (*CDH1*, *OCLN*, *DSP*) was observed in all treatment groups, while mRNA expression level of mesenchymal marker genes (*CDH2*, *FN1*, *VIM*) and EMT-related transcription factors (*SNAI1*, *ZEB2*, *TWIST1*) varied among treatment groups. TGF-β1 treatment induced the most pronounced increase in the level of mRNA of genes characteristic for mesenchymal phenotype that was accompanied with corresponding increase in protein level of two mesenchymal markers (*CDH2*, *FN1*), in parallel with decrease in protein expression of two epithelial markers (*CDH1*, *DSP*). To conclude, HCjE cells are prone to EMT induction and TGF-β1 possesses the highest potential for EMT induction in HCjE cells, suggesting that, in conditions of chronic inflammation, induction of EMT in conjunctival cells could contribute to fibrosis-related eye diseases.

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Developing a novel protein tagging, immunodetection and purification system

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Over the past years our laboratory has produced several monoclonal antibodies (mAb) that specifically recognize the p54 protein, the polyubiquitin receptor subunit of the 26S proteasome. We found that anti-p54 mAb1 recognizes the C-terminal region of the protein with unusually high specificity. Preliminary results suggested that the epitope of the antibody is relatively short, hence, it can be used to generate an epitope-tagging system for protein labelling, detection or purification. Therefore, we mapped the epitope of anti-p54 mAb1 and found that an 8-mer sequence localized in the carboxyl-terminal half of the p54 protein serves as the epitope of the mAb1.Then we started to examine model proteins tagged with the 8-mer epitope either on their N- or C-termini. We found that the 8-mer epitope served as an immuno tag since all chimeric model proteins were recognized by the anti-p54 mAb1 in Western-blot experiments. During this study we generated plasmid vector systems in which any gene of interest can be fused to the 8-mer epitope (N- or C-terminally) and expressed in bacteria or *Eukaryotic* cells in a constitutive manner or under the regulation of inducible promoters. Such expression system would allow us to label proteins with the 8-mer epitope for immuno-detection, staining or purification from bacteria to tissue culture cells.

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Effect of dietary trans fatty acids on ceramide and diglyceride levels in rat insulinoma cells

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Deleterious effects of permanently high free fatty acid levels are harmful to many cell types including the insulin producing pancreatic β-cells [1]. This lipotoxicity often causes ER stress and apoptosis due to an increased intracellular acyl-CoA supply and the consequent accumulation of biosynthetic lipid intermediates, such as ceramides and diglycerides. While the toxicity of the saturated palmitate (16:0) and the cis-unsaturated oleate (18:1 cis-Δ^9) has been widely investigated[2], limited data is available on the cell damages caused by elaidate (18:1 trans-Δ^9) and vaccenate (18:1 trans-Δ^{11}), despite the fact that the potential health effects of these dietary trans fatty acids (TFAs) received great publicity. We aimed to compare the effects of these four fatty acids at high concentrations (250-500 μM) on cell viability, apoptosis, ER stress, JNK phosphorylation and autophagy in RINm5F insulinoma cells. Changes in the overall fatty acid profile and the ceramide and diglyceride contents of the cells were also determined by using GC-FID and LC-MS/MS analysis. In accordance with earlier findings, we observed a marked toxicity of palmitate, which was attenuated by a simultaneous addition of oleate [2]. The two TFAs were found to be scarcely toxic in our experiments. Similarly to oleate and unlike palmitate, they reduced cell viability only at higher concentration, and their impact on ER stress, apoptosis and autophagy was not significant. Palmitate also caused a several fold increase in both ceramide and diglyceride levels, while much smaller elevations were induced by the unsaturated fatty acids, either cis or trans; however, the incorporation of TFAs in ceramides was strikingly more pronounced compared to oleate. These results show an obvious correlation between the severity of cell damage and the accumulation of lipid intermediates caused by different fatty acid species. This study on cellular effects of TFAs does not support a short term toxicity of these dietary compounds in insulinoma cells; nevertheless, it revealed some metabolic characteristics that might underlie a long term toxicity and hence deserve further investigation.

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In silico comparative analysis of cholesterol oxidases

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Apart from importance for animal cell membrane structure, cholesterol serves as a precursor for the synthesis of steroid hormones, bile acids, vitamin D and other bioactive substances in the human body. The determination of serum cholesterol is used in diagnostics for the assessment of atherosclerosis or coronary heart disease, estimating the risk of thrombosis and cardiovascular disease. Cholesterol oxidase is a group of enzymes that catalyzes conversion of cholesterol to cholest-4-en-3-one [1]. Cholesterol oxidases are widely employed by laboratories for the determination of cholesterol concentrations in clinical samples and food, in enzyme-assisted derivatization for sterol analysis (EADSA) in combination with LC-ESI-MS analysis [2,3]. The goal of this work was to create a reliable molecular dynamics models of the expressed cholesterol oxidases from *Pseudomonas* and *Streptomyces* genera with appropriate cofactor state and describe ligand behavior inside the active site of the enzyme. As a result, we presented a reasonable models of cholesterol oxidases. MD trajectories studies revealed significant diversity in the active sites of the proteins. Docking and molecular dynamics of the ligands bonded to the active sites of the molecules show the “hot points” of enzymatic bounding and reaction.

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Fatty acid profiling in cultured cells by using gas chromatography – flame ionization detection

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Oversupply of free fatty acids (FAs) stimulates both lipid biosynthesis and FA degradation. It has been repeatedly demonstrated that saturated FAs (e.g. palmitate) are far more deleterious than the endogenous cis-unsaturated FAs (e.g. oleate) or even the dietary trans-unsaturated FAs (e.g. elaidate and vaccenate) [1]. Increase in the level of saturation of membrane lipids has been reported to trigger the ER stress, which in turn may cause cell death and inflammation [2]. These observations highlight the importance of a balanced availability of saturated and unsaturated FAs in the cells. We demonstrate the development of a simple and validated method of gas chromatography with flame ionization detection for the quantification of 10 saturated and unsaturated FAs. The sample preparation uses a fast one-step reaction avoiding the use of chloroform. Calibration was linear between 0-200 µg/ml for each FAs with an R^2 >0.99. Recovery was 82% for unesterified FAs and >95% for complex lipids such as ceramides, diglycerides and triglycerides. LOD and LOQ were below 0.5 µg/ml. Robust method precision were achieved (RSD% was below 6% for each lipid classes). The method was tested on palmitate- and oleate-treated rat insulinoma cells. Our results show a significant shift in the ratio of the unsaturated and saturated FA content of the cells. The presented technique can be further developed to measure other intermediates of lipid biosynthesis and degradation as well.

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Antioxidant and cytotoxic activities of rosmarinic acid-rich *Salvia pratensis* L. extracts

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Salvia pratensis (meadow sage) is a flowering plant from the family Lamiaceae, native to Europe. Most of *Salvia* species have been widely used in the food, fragrance and drug industry, however, there is scarce literature data about *S. pratensis* investigations.[1] This study aimed to examine the phenolic profile of *S. pratensis* aerial part (SPA) and root (SPR) methanol extracts, their antioxidant potential (DPPH, ABTS, total antioxidant capacity and reducing power) and cytotoxic activity on two types of immortalized cells (HaCaT and BALB/c-3T3) and two cancer cell lines (A431 and SVT2). HPLC-PDA analysis of extracts s showed that rosmarinic acid was present in high concentration in both extracts. However, root extract also contained an unidentified compound in high concentration. The extracts showed significant antioxidant potential, especially antiradical effects with IC_{50} values ranging from 24-90 μg/mL. Based on the MTT reduction assay, an indicator of metabolically active cells, it can be noticed that the SPR was cytotoxic on all cell lines analyzed, whereas SPA was much less toxic, with no selective activity between normal and cancer cells. Further investigations will be focused on the identification of active constituents in *S. pratensis* root extract and their molecular mechanisms of action.

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Dopamine-modified pectins for laccase induced hydrogel formation and immobilization

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Pectins belong to a group of a plant polysaccharides that are structural components of plant cell walls. These polysaccharides have big potential for use in the food industry as a gelling agents and biomedical application due their biocompatibility, biodegradability, low price, etc [1]. Chemical modifications of pectin are useful methods for introducing various functional groups, which give pectin hydrogels novel properties. In this study, pectin was modified by oxidation with sodium periodate at molar ratios of 5, 10 and 15 mol% and reductive amination with dopamine and sodium cyanoborohydride afterwards [2]. This modification of pectin was confirmed by UV-VIS and NMR spectroscopy. Dopamine-pectins showed gelling properties in the presence of laccase and oxygen. These pectin derivatives were tested as carriers for laccase immobilization within microbeads formed in an emulsion based enzymatic polymerization reaction. Laccase was expressed in functional form in *E. coli*, isolated and additionally purified. We determined optimal conditions for immobilization andobtained microbeads with specific activity of 0.0006 IU/mg and 40% yield of immobilization. The immobilized laccase could be used for various application, such as decolorization of textile dyes [3].

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The effect of the extraction techniques on the antioxidant and antimicrobial activity of ethanolic extracts from sour cherry (*Prunus cerasus* L.) pedicles

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There is an increasing number of scientific investigations to find natural products that exhibit different biological activities, and antioxidant and antimicrobial activities are the most commonly studied. Sour cherry (*Prunus cerasus* L., Rosaceae) is a medicinal plant widely used in Ayurvedic medicine [1]. This plant is used in urinary system treatments to cure a number of diseases such as urinary tract infection, nephrolithiasis, cystolithiasis, and dysuria. Sour cherries contain phenolic components that possess many biological activities, such as antioxidant, anticancer, anti-inflammatory and antidiabetic properties [2,3]. Sour cherry fruits represent a significant source of health beneficial compounds widely used in the food industry. However, other plant parts, such as pedicles, have not been investigated yet. Therefore, the objective of the present study was to examine the effect of three extraction techniques (maceration, reflux extraction, and ultrasonic extraction) on the antioxidant and antimicrobial activity of ethanolic extracts from sour cherry (variety Oblačinska) pedicles. Extracts were obtained by maceration and ultrasonic extraction at room temperature during 120 minutes both, as well as by reflux extraction on boiling temperature during 120 minutes, by using 70% v/v ethanol, with solvomodulus 1:20 m/v. Antioxidant activity of extracts was determined spectrophotometrically by the DPPH test. The total phenolic content was determined using the Folin-Ciocalteu assay and the total flavonoids content was measured by a spectrophotometric method using AlCl3 reagent. Disc-diffusion method was used to determine antimicrobial activity on following pathogenic microorganisms: *E. coli, P. vulgaris, K. pneumoniae, B. subtilis, L. monocitogenes, S. aureus*, and *C. albicans*. The highest content of total phenolics (233.5 mg GAE/g of dry extract) was obtained by reflux extraction while the highest total flavonoids (15.25 mg RAE/g of dry extract) was obtained by ultrasonic extraction. The best antioxidant activity (EC50 value was 0.014 mg/cm3) showed the extract obtained by reflux extraction. The extract obtained by maceration showed antimicrobial activity on S. aureus and C. albicans while extract obtained by reflux extraction showed antimicrobial activity on *E. coli, P. vulgaris, B. subtilis, S. aureus,* and *C. albicans*. In the same time, the extract obtained by ultrasonic extraction showed no activity on tested microorganisms. The obtained results showed that the antioxidant and antimicrobial activity of sour cherry ethanolic extracts depends on the extraction technique applied. Also, the results indicated

that sour cherry pedicles could be used as resources for the isolation of natural antioxidants and antimicrobial agents as a safer alternative to synthetic additives.

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Recombinant expression and purification of yeast Frq1 protein

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Frequenin (Frq1) is a member of the NCS family of neuronal calcium sensor proteins. The Frq1 gene, encodes a protein of about 22 kDa in size, and plays essential roles in the yeast *Saccharomyces cerevisiae*, including regulation of growth and regulation of the activity of phosphatidylinositol-4-OH kinase (Pik1). Frq1 shares 60% sequence homology at the amino acid level with a mammalian protein called Neuronal calcium sensor-1 (NCS1). One of the most important roles of NCS1 protein in humans is regulation of neurotransmitter exocytosis, and movement of different proteins through the cell. These functions may be regulated by ligand binding, including lipids such as ceramides. To analyze interactions between Frq1 and ceramides, we first optimized recombinant expression and purification of the protein. Competent DH5-alpha *E.coli* cells were transformed with previously prepared plasmid DNA encoding Frq1. For expression purposes, BL21 competent bacterial cells were transformed with plasmid DNA isolated and purified from DH5-alpha cells. Isolation and purification of recombinant Frq1 was accomplished using immobilized nickel-affinity and size-exclusion chromatography. Chromatographic fractions were analyzed using SDS-PAGE electrophoresis. Results show that a significant quantity of Frq1 was expressed, and that the protein was stable throughout the purification procedure. Frq1 was obtained in amounts and purity sufficient for future research.

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The potential role of lead and selenium in pathogenesis of colloid goiter disease

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The thyroid gland is a well perfused endocrine organ and, consequently, appropriate target to the negative effects of metals (endocrine disruption) [1]. Colloid goiter (CG) represents the most common benign thyroid disease [2]. In contrast to endemic goiter, iodine deficiency has not been recognized as a factor for CG formation. The pathogenesis of nodular formation is unknown. Latest literature data indicated that environmental factors could contribute to nodular formation [3-5]. The aim of this study was to determine important toxic and essential trace metals in the goiter tissues (GTs). A further aim was to compare GTs (n = 41) to sex- and age-matched malignant thyroid tissues (MTTs, n = 40) and healthy thyroid tissues (HTTs, n = 40), in order to find the most significant metal or its ratio with other metal, that could play a key role in the pathogenesis of CG disease. Tissue samples were collected after surgery, and microwave digestion was applied for decomposition of samples. The concentrations of metals were determined by ICP-MS. Uni- and multivariate statistical methods were applied for data analysis. It was found that GTs had deregulated content of metals compared to the HTTs and MTTs. The lower content of essential metals (Mn and Se) and higher content of toxic metals (Pb, Th, and U) was found in GTs compared to the HTTs. The same toxic metals discriminated GTs from the MTTs by their higher contents, as well as Mn, Cd, and Cu/Zn ratio by their lower contents ($p < 0.05$). In order to establish more reliable criteria for separation of GTs from HTTs and MTTs, PCA was applied. The first PCA model explained 75.37% and the second PCA model explained 70.61% of a total data variance, respectively. The loading plot of the first model revealed that the most significant parameters discriminating HTTs from GTs were Mn and Se, indicating its evaluated content in HTTs, while the GTs showed evaluated contents of the U, Pb/Mn, and Pb/Se. Second PCA was applied to get criteria for separation of GTs from the MTTs. The main parameters that separated GTs from MTTs were Pb, Th, U, and

Pb/Se, while the contents of Mn, Cd, Cd/Se, and Cu/Zn significantly affect the differentiation of MTTs. Obtained results were in agreement with Mann–Whitney U-test. This study demonstrated, for the first time, that Pb could act as the main goitrogen, which could highlight its role in the unknown etiology of CG disease. Pb/Se could be considered as a relevant parameter for the separation of GTs from HTTs and/or MTTs. The obtained negative correlation between Pb and Se in GTs could explain the excision of Se by the high content of Pb.

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Phase separation, an important level of gene expression regulation

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Recently, liquid-liquid phase separation of proteins has become well known as an important step in the regulation of gene expression. Liquid-liquid phase separation of proteins is a specific form of phase transition in which soluble proteins reaching a critical concentration spontaneously separate into two adjacent liquid phases.A gel-like droplet is formed within the cytoplasm with a very high concentration of phase-separated protein, which results in a low protein concentration inthe remainder of the cytoplasm [1]. According to our working hypothesis, by phase separation of ribosome-nascent protein complexes cells can respond extremely quickly and efficiently to external or internal stress effects. The first stress effect initiates the expression of the complete gene regulatory cascade involved in the defense against stress. However, if the stress impact is over, the expressed mRNA-s are not degraded but phase-separated with the translating ribosomes to achieve a more rapid response against a second similar stress. After the second stress effect, only translation needs to be completed which has a huge evolutionary benefit, especially in caseof DNA damaging stress. We examined the negative effect of 1,6-Hexanediol on phase separation in yeast. 1,6-Hexanediol has been shown to inhibit the phase transition of proteins [2]. We have proved that the damaging effect of repeated UV treatment is increased by 1,6-Hexanediol, thus our hypothesis seems to be confirmed. We put forward the idea that phase separation may be the cause of resistance of certain cancer cells to radiotherapy.

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Inhibition of microsomal cortisol production – different polyphenols, different mechanisms

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Conversion of cortisone to cortisol by 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) in the endoplasmic reticulum (ER) of the target tissues is a major determinant of glucocorticoid action. The enzyme is functionally coupled with both hexose 6-phosphate dehydrogenase and glucose 6-phosphate translocase. Disorders of this peripheral hormone metabolism have been implicated in the pathomechanism of obesity-related metabolic diseases, such as obesity or type 2 diabetes mellitus; therefore, influencing this process can have preventive or therapeutic significance. Resveratrol and epigallocatechin gallate (EGCG) are polyphenols ofanti-obesity and anti-diabetic effects and unresolvedmolecular mechanisms. Their effects on the hepatic microsomal cortisol producing machinery was compared. Both polyphenols were found to reduce cortisol production in intact microsomal vesicles efficiently and in a concentration dependent manner. However, resveratrolachieved this effect by inhibiting 11βHSD1 directly, while EGCG interfered with 11βHSD1 activity indirectly, through anoxidative shiftin the ER luminal NADPH - $NADP^+$ pool. Our findings suggest that repression of prereceptor cortisol production likely contributes to the beneficial health effects of both resveratrol and EGCG once consumed regularly. Nevertheless, the exact molecular mechanisms of this action of polyphenolscannot be generalized. The failure of resveratrol to induce the redox shift, which was observed in case of EGCG, further supports the enzymatic nature of the underlying chemical reaction.As the two investigated natural polyphenols exert the samephysiological effect via separate modes of action, a combinational treatment in obesity-related diseases might result in a synergism without compromised tolerability.

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Selection of human haptoglobin alpha specific aptamers

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Haptoglobin is a serum glycoprotein, which usually forms a tetramer or higher order polymer structures from the disulphide bond linked alpha and beta subunits. Our collaborators have found that during *in vitro* fertilisation there is a proportional relationship between the level of the reduced haptoglobin alpha-1 fragment of oocyte culture medium and the viability of embryos [1]. Our primary goal was to generate aptamers which specifically recognise the above mentioned molecule; thus, they could be suitable for selective detection of the target protein in a complex matrix. Aptamers are short single stranded oligonucleotides with selective and binding affinity of those of antibodies but superior to antibodies in many aspects. For the aptamer selection, we performed the so called SELEX procedure. The target molecule haptoglobin alpha-1 was produced in bacterial expression system. Five cycles of selection were done using the purified, magnetic bead immobilised haptoglobin alpha-1 and an ssDNA library of random sequences. The selection was performed in the culture medium of embryo propagation, the so called G1 buffer. To identify the most prosperous aptamer candidates, dot blot assays were performed by using FAM labeled oligonucleotides. To this end, the asymmetric PCR produced, labeled oligonucleotides were incubated with magnetic-bead coated with haptoglobin alpha-1. Next, the specifically bound ssDNA were eluted, transferred to Hybond membrane, and fluorescence signal was detected. The most promising three oligonucleotides were chemically synthesized and presently are under thorough characterization to identify the aptamers which can be used for measuring haptoglobin alpha-1 in the culture medium.

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Determination of serum hypoxia-inducible factor-1: faster, reliable and accurate measurements with a new electrochemical impedance spectroscopy (EIS) based biosensor system

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Normal oxygen delivery is essential for survival. Hypoxia, which is a common feature of various pathological conditions, ranging from cancer to inflammatory diseases, occurs when normal oxygen delivery is altered by an imbalance between cellular oxygen demand and tissue oxygen supply. Among the intricate mechanisms organisms have developed to maintain oxygen homeostasis, a family of hypoxia-inducible transcription factors (HIFs), are found to be the main regulator adaptive cellular response to hypoxia. Although ELISA can be used for its measurement, the lability of the protein and length of the analysis (5 h) pose limitations. Thus, our aim is to develop an electrochemical impedance spectroscopy (EIS) based biosensor system for quick and reliable measurement of HIF-1α in serum. HIF-1 α antibodies have been used as a biota receptor. For immobilization, the electrode was first modified with albumin, followed by PAMAM. The new biosensor was compared with the conventional ELISA method. Based on the chronoimpedance data, total analysis time for EIS was chosen as 15 min. Calibration curve was constructed by locating electron transfer resistance on y-axis and HIF1 concentration on x-axis, between 50-1000 pg/mL. LOD and LOQ of the biosensor were calculated as 14.45 pg/mL and 43.80 pg/mL, respectively. The new biosensor showed very good correlation when compared with the conventional ELISA method (R^2= 0.99649). We developed and analytically validated a biosensor system to measure HIF-1α in serum. This new biosensor promises more timely and accurate measurements in determining the tissue oxygenation in patients who have hypoxia related conditions such as diabetic foot.

The effect of buffer composition and nonionic surfactants on trypsin cold stability

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Trypsin is a serine protease with applications such as protein sequencing and tissue dissociation. It was shown that the storage of trypsin in acidic conditions affects the activity recovery by more than 40% after 7 freeze-thaw cycles and that the cold storage in ammonium-bicarbonate with the addition of cryoprotectants glycerol or lysine leads to the protein stabilization and high activity recovery[1]. In present study, potassium-phosphate and ammonium-phosphate buffers were used with 90% and 70% activity recovery rate after 7 freeze-thaw cycles, respectively. Further, nonionic surfactants (NS) - Tween 20, Tween 80 and Triton X-100 were used with 94%, 89% and 63% activity recovery rate. Changes of bands maxima corresponding to specific secondary structures in the FT-IR spectrum were insignificant which is indicative of no freezing-induced denaturation of the trypsin protein. Denaturation of trypsin occurs when the protein adsorbs to the ice crystal surface during the crystallization process. NS's can protect trypsin by competing with the protein for sites on the ice surface. Trypsin stored in potassium-phosphate with NS's, which was used for digestion in the conventional method for preparing *Listeria monocytogenes* suspension from meat products, significantly improved the recovery yield as evidenced by an increase in the number of *L. monocytogenes* colony forming units.

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Protein homeostasis is maintained by the gene expression circuitry

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In the past decade several mechanisms were discovered that connects different gene expression steps [1-3]. The Ccr4-Not complex is a conserved multi-protein complex that regulates gene expression at all stages, from production of the mRNA in the nucleus to its degradation in the cytoplasm. Challenging the general understanding of gene expression that considers transcription and translation to be independent processes, we recently demonstrated that translation efficiency is determined during transcription elongation through imprinting of ribosomal protein mRNAs with Not1, the central scaffold of Ccr4-Not [3]. We also determined that Not5-dependent Not1 association with specific mRNAs was important during translation for assembly of protein complexes such as RNA polymerase II, the SAGA histone acetyltransferase and the proteasome [4-6]. This regulation of transcription during translation and translation during transcription places the Ccr4-Not complex at the core of the gene expression circuitry. I will summarize published and ongoing work about the functions of the Ccr4-Not complex that we identified in yeast and I will discuss how I think these might be relevant to understand why Ccr4-Not and ribosome protein mutations accumulate in numerous cancers.

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Differential secretion of MIF in normal and transformed human trophoblast cell lines

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Macrophage migration inhibitory factor (MIF) is a multifunctional cytokine expressed by various cell types including trophoblast. MIF participates in regulation of trophoblast migration and invasion, crucial processes in development of placenta and establishment of normal pregnancy [1]. MIF can act in autocrine and paracrine manner binding to its receptors. We showed previously that expression of MIF receptor CD74 is significantly higher in transformed compared to normal trophoblast cells. In this study differential expression of noncognate MIF receptor CXCR2 and secretion of MIF in immortalized first trimester of pregnancy extravillous trophoblast cells HTR-8/SVneo and choriocarcinoma cell lines JAr and Jeg-3 was examined. Our results showed that CXCR2 expression is significantly higher in Jeg-3 cells compared to the HTR-8/SVneo cells. There was no significant difference of MIF protein expression in whole cell lysates of the three cell types studied. However, the level of secreted MIF was significantly lower in conditioned media of JAr cell line compared to both HTR-8/SVneo and Jeg-3 cells. Furthermore, treatment of estradiol producing JAr cells with estradiol receptor antagonist Fulvestrant led to a significant increase in MIF secretion which is in line with literature data regarding regulation of MIF secretion by estradiol [2]. Differential expression of noncognate MIF receptor CXCR2 and differences in MIF secretion between normal and transformed trophoblast cell lines indicate that MIF may be involved in physiology of transformed trophoblast.

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Determination of antithyroglobulin antibodies concentration in human serum using Quartz Crystal Microbalance sensors

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Determination of concentration of antithyroglobulin antibodies (TgAB) is one of the main methods used in diagnostics of the diseases of the thyroid gland, such as Hashimoto thyroiditis, Graves disease, and some types of thyroid gland cancers [1]. Assays for determination of concentration of these antibodies which are currently used in diagnostics require reagent labelling, a time consuming process with associated financial costs. In the present day, there is a big demand for fast, sensitive and label-free assays for the determination of concentration of many clinically important compounds. Quartz Crystal Microbalance (QCM) technology allows the development of such assays, and assays for the determination of concentration of some proteins have already been developed [2,3]. So far, no one has developed an assay based on QCM technology for the determination of concentration of TgAb in human serum. Because the determination of concentration of TgAb in human serum plays an important role in diagnostics, we immobilized thyroglobulin on the surface of Attana LNB Carboxyl sensor chip. After that we prepared standard curve for the determination of concentration TgAb in patients' samples within range of 1-50000 kIU/L, and established optimal experimental conditions. Results obtained for patients' samples were compared to the results from the reference laboratory at Institute for Application for Nuclear Energy. Results obtained with QCM-based assay were very similar to the results obtained in the reference laboratory which uses radioimmuno assay, especially for clinically relevant values of TgAb concentration. It was also shown that standard control human serum must be used for preparation of standard curve.

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Oxidized LDL in obese children and children with type 1 diabetes mellitus

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Oxidative stress (OS), dyslipidemia and inflammation have an integrative role in the development of cardiovascular complications in obese and patients with type 1 diabetes mellitus (T1DM)[1]. Cumulative evidence suggests that oxidative modification of low-density lipoprotein (LDL) particles could be a common mechanism of increased cardiometabolic risk in obesity and T1DM1[1,2]. However, little is known about its significance in the pediatric population[3]. The aim of our study was to investigate the potential value of oxidized LDL (oxLDL) determination, as a biomarker of OS, in obese and children with T1DM, and also to examine the relationship between the biomarkers of OS, dyslipidemia and inflammation in these patients. For this purpose, oxLDL, advanced oxidation protein products (AOPP), prooxidant-antioxidant balance (PAB), high-sensitivity C-reactive protein (hsCRP) and lipid status parameters were measured in 26 normal weight children, 74 obese children and 74 children with T1DM. Both, obese and T1DM children had significantly higher levels of OS markers than their normal weight peers. The patients with T1DM had more favourable lipid profile compared to obese children, as reflected by lower triglycerides ($P<0.05$) and LDL-cholesterol ($P<0.05$) levels and higher high-density lipoprotein (HDL)-cholesterol concentration ($P<0.001$). Yet, the levels of OS markers, AOPP ($P=0.058$) and PAB ($P<0.05$), and hsCRP were higher in T1DM patients, than in obese children. Moreover, the level of oxLDL in T1DM group (median: 30.65 ng/mL; interquartile range: 24.72-36.27 ng/mL) was significantly higher ($P<0.001$) than the level in obese (median: 20.60 ng/mL; interquartile range: 13.12-29.82 ng/mL) and in normal weight children (median: 22.11 ng/mL; interquartile range: 15.67-35.20 ng/mL). Ordinal regression analysis was employed to assess the association between oxLDL level and cardiometabolic risk among examined categories of pediatric patients. In univariate analysis, an increase in oxLDL level was significantly associated with the higher probability that patient has increased weight or T1DM (OR: 1.03; 95%CI: 1.00-

1.06; P<0.05). The observed association remained significant after adjustment for age and gender (OR: 1.03; 95%CI: 1.00-1.05; P=0.05) and hsCRP levels (OR: 1.03; 95%CI: 1.00-1.06; P<0.05), and of borderline significance after adjustment for lipid status parameters (OR: 1.03; 95%CI: 1.00-1.05; P=0.056). In conclusion, oxLDL could be a valuable biomarker in the assessment of cardiometabolic risk in pediatric obese and T1DM patients.

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Testing the potential rate-limiting role of electron transfer proteins in fatty acid desaturation

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The stress caused by permanently elevated fatty acid (FA) levels can lead to cellular dysfunction or even cell death, which contributes to the development of pathological conditions, such as cardiovascular diseases, non-alcoholic fatty liver disease and type 2 diabetes [1]. The most severe lipotoxicity is caused by an unbalanced oversupply of saturated FAs (e.g. palmitate). Unsaturated FAs (*e.g.* oleate) are less toxic, moreover they can reduce the damage caused by saturated ones, and this highlights acyl-CoA desaturation as a cellular defense mechanism and hence the efficiency of stearoyl-CoA desaturase (Scd1) is an important factor of resistance [2]. A novel oxidoreductase has been shown to protect cells against palmitate toxicity, so we aimed to test whether Scd1 itself or the associated electron supply is rate-limiting for cellular desaturase activity. The FA profile was assessed by GC-FID analysis in transiently transfected HEK293T cells. Overexpression of Scd1 resulted in a marked elevation of unsaturated/saturated FA ratio, but this effect was not achieved by overexpression of the Scd1-related electron transfer proteins. The electron supply did not become rate-limiting even in palmitate-treated cells or in cells of enhanced Scd1 expression and activity. In accordance with other observations, our findings indicate that Scd1 enzyme itself catalyzes the rate-limiting step of FA desaturation, and this function cannot be facilitated by reinforcing the electron supply of the enzyme in this cell line.

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