Prof. Krzysztof W. Szewczyk

VIIIth Intercollegiate Biotechnology Symposium

SYMBIOZA

BOOK of ABSTRACTS

17th-19th May 2019

Warsaw, Poland

VIIIth Intercollegiate Biotechnology
Symposium “Symbioza”
17-19 May 2019

msb.symbioza.edu.pl

Book of abstracts

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Welcome message

Dear 8th Symbioza participants!

As you are reading these words, you have successfully arrived at the Intercollegiate Biotechnology Symposium "Symbioza". I am delighted beyond words that you have decided to dedicate this weekend to a joint explorative venture into science.

This year will be the eighth time this event will take place; in chemistry the number eight is associated with oxygen, and what with biology? The first organism that came to our mind was the octopus! And thus the mollusc became our annual mascot representative. I encourage you to wear with pride, gadgets featuring our one and only Doctopus!

Before you lay an array of fascinating lectures, brought from all over the world, as well as the opportunity to see the fruits of our friends' work. The topic variety is encompassing enough that there is something for everyone to discover.

Touched by Feynman's words: *"I don't know what's the matter with people: they don't learn by understanding, they learn by some other way — by rote or something. Their knowledge is so fragile!"*

Let us build our knowledge on the foundation of comprehension. Make the most of this time and ask questions! It is said that the utmost thorough means of understanding is through discourse, and I can't wait to have the chance to engage in heated discussion.

In the end however, there are things in life and in the world beside science. This year, for the first time, we have prepared for you 4 parallel workshop sessions to choose from. I hope that you will find something for yourself amongst Friday's activities: public presentations, running private enterprises, discussion session with one of our keynote speakers, or the "Pipetting Academy". And that you will have a pleasant opportunity to get to know each other during the Saturday get-together, where we will be able to apply our networking skills!

I hope that by the end of it, you will have a positive memory of Symbioza and that it will remain with you even until next year's registration.

On behalf of the organising team,

Patrycja Ściślewska,
President of IBS Symbioza

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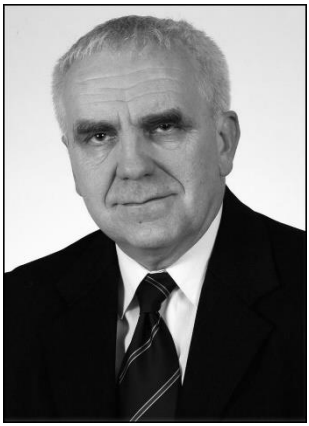
Patron of the conference



Prof. Krzysztof Włodzimierz Szewczyk

(1952-2011)

Professor Szewczyk was a remarkable scientist, well-recognized specialist in the field of industrial biotechnology and bioprocess engineering both in Poland and outside the country. He was a co-founder and organizer of Biotechnology studies at Warsaw University of Technology. Professor Szewczyk was also a director of Intercollegiate Biotechnology Centre at WUT (2007-2008) and a supervisor of the Department of Biotechnology and Bioprocess Engineering at the Faculty of Chemical and Process Engineering at WUT (2006). He also used to teach bioprocess engineering at University of Warsaw. He was known as an excellent and valued teacher among students, thus in 1995 he received the Silver Cross of Merit. Moreover, in 2003 he was awarded with the Commission of Education Medal and in 2008 distinguished with Ministry of Science and Higher Education Award. Since 2003 he had been a member of Committee of Biotechnology during the Presidium of Polish Academy of Sciences and in the years 1992-1995 the secretary of Bioprocess Engineering section in the Committee of Chemical Process Engineering at Polish Academy of Sciences. Furthermore, professor Szewczyk was a member of Programme Council of quarterly journal “Biotechnology” (2005-2010) and a Vice-President of Polish Federation of Biotechnology (2007-2010). He was an author of more than 120 scientific articles, co-author of 6 patents and utility designs and author or co-author of 8 student’s handbooks. Colleagues, professors and students remember him as an erudite, wed of classical music and chess enthusiast who was truly wedded to education among academic adolescents.

Honorary patronage

Honorary patronage

His Magnificence Rector of Warsaw University of Technology
Prof. **Jan Szmidt**, PhD, DSc

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Platinium sponsors

Roche

Roche is a global pioneer in pharmaceuticals and diagnostics focused on advancing science to improve people's lives. The combined strengths of pharmaceuticals and diagnostics under one roof have made Roche the leader in personalised healthcare – a strategy that aims to fit the right treatment to each patient in the best way possible.

Roche is the world's largest biotech company, with truly differentiated medicines in oncology, immunology, infectious diseases, ophthalmology and diseases of the central nervous system. Roche is also the world leader in *in vitro* diagnostics and tissue-based cancer diagnostics, and a frontrunner in diabetes management.

Founded in 1896, Roche continues to search for better ways to prevent, diagnose and treat diseases and make a sustainable contribution to society. The company also aims to improve patient access to medical innovations by working with all relevant stakeholders. Thirty medicines developed by Roche are included in the World Health Organization Model Lists of Essential Medicines, among them life-saving antibiotics, antimalarials and cancer medicines. Moreover, for the tenth consecutive year, Roche has been recognised as the most sustainable company in the Pharmaceuticals Industry by the Dow Jones Sustainability Indices (DJSI).

The Roche Group, headquartered in Basel, Switzerland, is active in over 100 countries and in 2018 employed about 94,000 people worldwide. In 2018, Roche invested CHF 11 billion in R&D and posted sales of CHF 56.8 billion. Genentech, in the United States, is a wholly owned member of the Roche Group. Roche is the majority shareholder in Chugai Pharmaceutical, Japan.

Sartorius

The Sartorius Group is a leading international partner of biopharmaceutical research and the industry. With innovative laboratory instruments and consumables, the Group's Lab Products & Services Division is a premium supplier of high-quality laboratory instruments, high-grade consumables and excellent services and concentrates on serving the needs of laboratories performing research and quality control at pharma and biopharma companies and those of academic research institutes. The Bioprocess Solutions Division with its broad product portfolio focusing on single-use solutions helps customers to manufacture biotech medications and vaccines safely and efficiently. The Group has been annually growing by double digits on average and has been regularly expanding its portfolio by acquisitions of complementary technologies. Currently, more than 8,100 people work at the Group's approximately 60 manufacturing and sales sites, serving customers around the globe.

Supporting institutions

Supporting institutions

Students' Union – Warsaw University of Technology

Students' Union – University of Warsaw

Students' Union – Warsaw University of Life Sciences

Polish Children and Youth Foundation

Organizers

The **Warsaw Society of Biotechnology 'Symbioza'** (WSB *'Symbioza')* was established in 2013, thanks to cooperation between students from three Warsaw universities: University of Warsaw, Warsaw University of Life Sciences and Warsaw University of Technology.

Symbioza unites people who want to share knowledge and experience with other young students and scientists coming from around the word. The main purpose of *Symbioza* is popularization of biotechnology by organizing many events such as:

- The main project of the society: **prof. Krzysztof W. Szewczyk Intercollegiate Biotechnology Symposium '*Symbioza*'** (*IBS 'Symbioza'*) – a scientific conference during which young scientists can meet and present their work. The symposium is addressed to students and PhD candidates from around the world who want to broaden their knowledge on bioscience. The first edition of IBS 'Symbioza' was appreciated in the STRUNA competition, 'Conference of the year 2012' category, winning the 2nd place.
- **OAK** (*Attractive Conventicles Camp*) – workshops that aim to improve the scientific presentation techniques and show new ways of transferring knowledge and presenting research results.
- **'Feel the Flow Project'** – series of meetings with famous science popularizes and workshops devoted to issues accompanying scientific work.
- Taking part in the **Scientific Picnic** co-organized by the Copernicus Science Centre during which *Symbioza* tries to explain the fascinating world of biotechnology to the youngest enthusiasts.

Scientific Committee

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SGGW	Warsaw University of Life Sciences
UW	University of Warsaw
PW	Warsaw University of Technology
IMDiK	Mossakowski Medical Research Centre Polish Academy of Sciences
WUM	Medical University of Warsaw
UJ	Jagiellonian University
IBA	Institute of Biotechnology and Antibiotics
CO-I	Maria Curie-Skłodowska Center of Oncology-Institute in Warsaw
Nencki	Nencki Institute of Experimental Biology of the Polish Academy of Sciences

Organising Committee

President of the Organising Committee

Patrycja Ściślewska

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Co-organizers

Biotechnology Student Interest Group "Herbion" was established in 2003 at the Faculty of Chemistry of Warsaw University of Technology. For all these years "Herbion" conveyed a number of scientific projects, from the construction of a bioreactor, through biotransformations using enzymes, creation of a Warsaw University of Technology perfume brand "Entropy", to genetically engineered phage of lactic acid bacteria. The Herbions, as the members proudly call themselves, popularized biotechnology through science shows and promoted the Faculty and the University during many mass events such as the Science Picnic of Polish Radio and the Copernicus Science Centre, the SIGs and Student Organizations' Fair, open days at Warsaw University of Technology and Academic Village during yearly student carnival (Juwenalia). Other activities include organizing many educational trips, such as nine Biotechnology Students Science Camps, excursions to the brewery in Żywiec, Lublin and Tychy, to Avon, or Dr. Irena Eris factories and even to Central Forensic Laboratory of Warsaw Metropolitan Police.

Student research group 'KNBiotech' was established in 1997 at the Faculty of Horticulture, Biotechnology and Landscape Architecture of Warsaw University of Life Sciences. It gathers students of biological studies interested in widely understood biotechnological science with goals such as:

- work on research projects (at University departments and institutes such as Institute of Biochemistry and Biophysics PAS, Nencki Institute of Experimental Biology PAS, The Maria Skłodowska-Curie Institute – Oncology Center)
- education of students (workshops, regular meetings "Feel the Flow" organized with Symbioza)
- popularization of science (Science Picnic of Polish Radio and Copernicus Science Center, Festival of Science and Warsaw University of Life Sciences Open Days)

KNBiotech is a unique place of intergenerational integration for every science enthusiast – from a student of first year to a distinguished professor. Passion, friendships and careers are born here.

Student Interest Group of Molecular Biology cooperates at the Faculty of Biology of University of Warsaw since 1995. The group's supervisors are outstanding geneticists and science promoters: prof. dr hab. Ewa Bartnik and prof. dr hab. Paweł Golik. The basis of activity are weekly meetings, at which students present seminars regarding wide range of topics related to molecular biology. The series of lectures with experts representing the best Warsaw research centers (such as Nencki Institute of Experimental Biology PAS, Medical University of Warsaw, Mossakowski Institute of Experimental and Clinical Medicine PAS) are organized. Members of group participate also in events popularizing science, such as Science Picnic or Biologists' Night. Moreover, every year Student Research Group of Molecular Biology organizes Student Research Groups Symposium at the Hyrobiological Station in Pilchy, where you can find perfect atmosphere for exchange of ideas between students and professors. This year's topic of the symposium is "From idea to innovation – application of basic research".

Programme

Friday (17th May)

13:00-15:00 **Registration**

15:00-15:15 **Opening ceremony**

15:15-16:05 **Plenary lecture** Room 107

Gretchen Edwalds-Gilbert
Regulation of gene expression by mRNA translation in response to stress

16:05-16:15 **Industry lecture** Room 107

Fulbright

16:15-16:35 **Industry lecture** Room 107

Roche

16:35-16:45 **Coffee break**

16:45-17:45 **Oral sessions:**

Student oral session 1 Room 107
Let the cat out of the bag

Agnieszka Chlebicz **O25**
Effect of sows feed supplementation with novel synbiotics on faecal concentrations of lactic acid and short-chain fatty acids

Grzegorz Suwala **O26**
Sex chromosomes in lacertid lizards: high stability in spite of reported variability

Alicja Nowacka **O27**
Interaction of serum-derived and internalized C3 with DNA in human B cells – a potential involvement in regulation of gene transcription

Aleksandra Fesiuk **O28**
Dysregulated miRNA in human plasma as potential biomarkers in Alzheimer's disease

Student oral session 2 Room 227
A prescription for transcription

Igor Grochowina **O17**
The interaction of Hsp70/Hsc20 chaperones with folded protein substrate

Piotr Soczewka **O18**
Yeast-model-based study identified myosin- and calcium-dependent calmodulin signalling as a potential target for drug intervention in chorea-acanthocytosis

Marcin Lipiec **O19**
The transcription factor TCF7L2 confers and maintains postmitotic neuronal identities in vertebrate thalamus and habenula

Klaudia Peczyk **O20**
Analysis of HvTIP1;1 gene expression coding for aquaporin in barley (Hordeum vulgare L.) after treatment of seedlings with compounds inducing oxidative stress

17:45-17:55 **Coffee break**

17:55-18:40 **Plenary lecture** Room 107

Paweł Sachadyn
Mammalian regeneration

18:45-19:45 **Workshops** Rooms: 107, 213, 214, 227

Saturday (18th May)

10:00-10:50 **Plenary lecture** Room 107

Radosław Zagożdżon
Immunotherapy with genetic engineering as the most promising approach to cancer treatment

10:50-11:05 **Industry lecture** Room 107

Sanofi

11:05-11:15 **Coffee break**

11:15-12:15 **Oral sessions:**

Student oral session 3 Room 107
How to cancel the cancer?

Malwina Sosnowska **O05**
Fullerenes triggered G2/M-phase arrest of liver cancer cells by mechanotransduction towards membrane proteins, cytoskeleton and the nucleus

Żaneta Kałuzińska **O06**
The regulation of AP2-α transcription factor via WWOX protein and its influence in bladder carcinogenesis

Łukasz Arcimowicz **O07**
Identification of molecular markers for detection and characterisation of single breast cancer circulating tumour cells in different epithelial-to-mesenchymal states

Maria Latacz **O08**
Rs10877012 polymorphism in CYP27B1 gene in women with diagnosed breast cancer: preliminary study

Student oral session 4 Room 227
ekoLOGIC

Joanna Jablonska **O09**
Heavy metal tolerance in bacteria isolated from soil contaminated with polycyclic aromatic hydrocarbons (PAHs)

Anna Litwin **O10**
The study of the accumulation of insecticides and the impact on cell permeability in entomopathogenic fungus Beauveria bassiana

Mateusz Grzybowski **O11**
Investigation of diversity of bacterial microflora in the stratosphere

Justyna Kajdanowicz **O12**
Analysis of antibiotic resistance of Staphylococcus spp. *strains isolated from pigs*

12:15-12:25 **Coffee break**

12:25-13:15 **Plenary lecture** Room 107

Thomas Cremer
Higher order chromatin organization and the interchromatin compartment - an integrated concept of the dynamics of nuclear architecture and function

13:15-13:35 **Industry lecture** Room 107

Labnatek

13:35-14:35 **Dinner**

14:35-15:35 **Poster session #1 and coffee** Ground floor

15:35-16:25 **Plenary lecture** Room 107

Barbara Kalinowska
Real life bioinformatics in life science industry

16:25-16:40 **Industry lecture** Room 107

Sartorius

16:40-16:50 **Coffee break**

16:50-17:50 **Oral sessions:**

Oral session 5 Room 107
Bio ≤ Technology

Klaudia Arciszewska **O13**
Anti-lysine DNA aptamer - a novel tool for purification of recombinant proteins

Milena Stolarska **O14**
Structural pre-adaptation and episodic selection drove evolution of moonlighting activity of Hsp70 co-chaperone Zuotin

Witold Postek **O15**
Microfluidic screening of antibiotic susceptibility at the single-cell level

Marcelina Jureczko **O16**
Potential removal of cytostatic pharmaceuticals by Tritirachium album - sorption study

Oral session 6 Room 227
Mystery of Biochemistry

Róża Szatkowska **O29**
What is the connection of tRNA synthesis with storage carbohydrates in Saccharomyces cerevisiae?

Małgorzata Milewska **O30**
Search for human proteins showing potential interactions with Toxocara canis CTL-1 antigen using yeast two hybrid system

Izabela Perkowska **O31**
Revealing the unknown. Discovering the biological function of selected plant UDPglucosyltransferases

Katarzyna Kluszczyńska **O32**
How not to purify exosomes?

18:00-19:30 **Evening walk around the Old Town**

21:00… **Social event at Iskra Club** Wawelska 5

Sunday (19th May)

10:00-11:00 **Oral sessions:**

Oral session 7 Room 107
Make it viral!

Izabela Serafińska **O21**
Potential of autophagy in equid herpesvirus type 1 (EHV-1) therapy

Łukasz Richter **O22**
Bacteriophages as a tool for rapid and sensitive bacteria detection

Agata Woźniak **O23**
All you need is light – significance of endogenous porphyrins presence in resensitization of Acinetobacter baumannii to antimicrobials

Karolina Paszkowska **O24**
The interaction between T4 bacteriophages and different polypropylene surfaces

Oral session 8 Room 227
Huge problems in nanoscale

Jarosław Szczepaniak **O01**
Effect of Reduced Graphene Oxides on Cell Membrane and Expression of Genes Related to the Functioning of Voltage Gated Ion Channels in Glioma Cells

Adrian Augustyniak **O02**
Regulatory effect of graphene oxide on secondary metabolism in streptomycetes

Bartłomiej Dominiak **O03**
Preliminary study on the usability of biodegradable ZnO nanoparticles doped with Fe for iron supplementation

Daria Ciecholewska **O04**
Nanomaterials based on bacterial cellulose with increased water absorption

11:00-11:10	**Coffee break**	
11:10-12:00	**Plenary lecture**	Room 107
	Egor Dzyubenko Studying neuroplasticity after stroke: how technologies pave the way for new ideas	
12:00-13:00	**Poster session #2 and coffee**	Ground floor
13:00-14:00	**Dinner**	
14:00-14:50	**Plenary lecture**	Room 107
	Alessio Mengoni *Toward an integrated and predictive view of symbiotic nitrogen fixation*	
14:50-15:30	**Closing ceremony**	Room 107

List of posters

Poster session I

18th May 2019 14:35-15:35

P01 **Luca Baiamonte**

Asymmetric flow field-flow fractionation
for the characterization of biologically active molecules and assemblies

P02 **Jaśmina Bałaban**

The effect of graphene oxide nanofilm on mitochondrial activity
and biogenesis in chicken embryo muscle progenitor cells

P03 **Anna M. Banaś**

Campylobacter jejuni C8J0565 plays a key role
in the defense against cooper and oxidative stress

P04 **Joanna Banot**

Evaluation of the membrane support influence
on adherent cell function in biohybrid system

P05 **Joanna Baran**

Behavior of melanoma cells with different metastatic potential
on surfaces modified with proteins from extracellular matrix

P06 **Małgorzata Ciężkowska**

C3A cell line with restored urea cycle
as a promising source of cells for BAL devices

P07 **Magdalena Damentko**

Quantitative analysis of Fe distribution in mouse tissues
after supplementation with ZnO: Fe nanoparticles,
comparison of Tissue-Facs and Micro Image methods

P08 **Anna Długajczyk**

Efficient vesicular trafficking protects yeast *Saccharomyces cerevisiae*
genome from fragmentation

P09 **Jan Długosz**

Western diet accelerates neuroinflammation
in mice model of Alzheimer's disease

P10 **Kamila Dubrowska**

Rapid identification of biofilm-forming
Staphylococcus pseudintermedius strains isolated from dogs

P11 **Magdalena Flont**

Analysis of anticancer drug cytotoxicity
on the spatial breast cancer model in the Lab-on-a-chip system

P12 **Marta Gołębiewska**

Safe and natural – antimicrobial compounds
produced by lactic acid bacteria against pathogenic *Escherichia coli*

P13 **Marcin Tomasz Gradowski**

Pathogenic *Legionella* protein kinase families

P14 **Ummi Hani**

Application conjugate of enkephalin and temporal DAL-PEG-DK5
in the treatment of periodontitis

P15 **Damian Kaniowski**

Conjugates of antisense oligonucleotides with boron clusters:
new material for Boron Neutron Capture Therapy

P16 **Anna Kobuszewska**

Lab-on-a-Chip system integrated with nanofibrous mats
for hypoxia symulation

P17 **Paweł Krzyżek**

3-Bromopyruvate – an anti-carcinogenic compound
with an activity against *Helicobacter pylori*

P18 **Agata Leśniewska**

Immune escape and stem cell markers profile of breast cancer cell lines
in the context of epithelial-to-mesenchymal transition

P19 **Iwona Lewandowska**

Application of *in silico* methods in structure analysis of supramolecular
complexes of cyclodextrins with active pharmaceutical ingredients used
in the treatment of pulmonary hypertension

P20 **Przemysław Liczbiński**

Escherichia coli biofilms and their enzymatic fingerprinting

P21 **Kamila Liman**

Stereoselective reduction of monohydroxy flavanones
to corresponding flavan-4-ols by the yeast *Rhodotorula glutinis* KCh735

P22 **Aneta Lukasiewicz**

Preliminary analysis of transcriptome indicates potential mechanisms involved in carrot response to salt stress

P23 **Michał A. Małszycki**

Phylogeny of type I and II J Domain Proteins
using Bayesian and Maximum Likelihood methods

P24 **Tomasz Obrębski**

On the trail of new markers of pacemaker cells

P25 **Oleksandra Oskyrko**

Distribution of lacertidae in the danube – dniester region (Ukraine)

P26 **Marta Pałka**

Defining lamin-associated proteome in *Drosophila melanogaster*
- a new approach to the molecular basis of laminopathies

P27 **Maciej Prusinowski**

Interactions of recombinant TRF1 and TRF2 shelterine proteins
with telomeric DNA.

P28 **Mateusz Przetocki**

Mechanical properties of erythrocytes treated with multi-walled carbon nanotubes functionalized with carboxyl groups

P29 **Katarzyna Przygrodzka**

Synthesis and analysis of operational parameters
the Nickel Ferrite- Cross-Linked Enzyme Aggregates (NiFe-CLEAs)
of glucoamylase and lipase

P30 **Aleksandra Rapacka- Zdończyk**

Multiple sub-lethal antimicrobial photodynamic inactivation
and ciprofloxacin treatments lead to *S. aureus* sensitization
to gentamycin and doxycyclin

P31 **Katarzyna Ratajczak**

Utilization of oligonucleotide molecular beacon probe
on graphene oxide nanocarrier for the detection
of survivin mRNA in colorectal cancer cells

P32 **Mariia Savchenko**

Searching for the right model:
environmental niche modelling of one bat species

P33 **Sandra Skorupska**

New electrode modules application for cell electroporation

P34 **Dominika Stradomska**

Dynamic kinetic resolution of primary amines using rotating bed reactor

P35 **Grzegorz Suwala**

Fine-tuning of the Z chromosome gene content in lacertids

P36 **Jordan Sycz**

Microbial modification of pregnenolone
by entomopathogenic fungi *Isaraia farinosa*

P37 **Barbara Szmulkowska**

Resistance phenotypes of *Listeria* spp. strains
isolated from soil environments

P38 **Patryk Sztandarski,**

Detection of the CC398 clonal complex S*taphylococcus aureus*
isolated from horses

P39 **Adam Szymajda**

The elusive perpetrators
– high-throughput sequencing in search of photos destroyers

P40 **Wiktor Tokarek**

The violaxanthin cycle and the greening process
in young wheat seedlings

P41 **Aleksandra Tomczak**

Drosophila melanogaster – an excellent model
for studying stress response and its impact on the cell nucleus

P42 **Marcin Tymiński**

Impact of cold stratification of apple (*Malus domestica* Borkh.) seeds
on malondialdehyde (MDA), phenols content
and total antioxidant activity in embryonic axes

P43 **Marta Wiatrowska**

Effect of a prior experience
in the model of socially transferred fear in Wistar rats

P44 **Aleksandra Wielento**

Citrullinated *Porphyromonas gingivalis* surface proteins
as a crucial factor enhancing inflammation
in human gingival fibroblasts and their differential response

P45 **Patrycja Wojtaczka**

One-step purification and kinetic analysis
of phospholipase A2 from the venom of *Vipera wagneri*

P46 **Norbert Wroński**

Nanosilica from biomass for heavy metal removal

P47 **Anna Sobiepanek**

The elaboration of an additional melanoma diagnostic procedure
based on label-free methods

P48 **Camilla Fagorzi**

Exploiting pangenomes: comparative genomics
and deep phenotyping of the plant-associated genus Ensifer

P49 **Angelika Michalak**

Better together. Silver nanoparticles, *Iris pseudacorus* plant extracts
and their synergistic interaction act against bacterial human pathogens.

Poster session 2

19th May 2019 12:00-13:00

P50 **Karolina Bańkowska**

Modulation of daunomycin biological activity by silver nanoparticles

P51 **Piotr Bartosz**

New way to fight *Staphylococcus aureus* wound infections

P52 **Carlo Bieńkowski**

The clinical course of *Listeria monocytogenes* meningitis compared to other community-acquired bacterial meningitis

P53 **Paulina Błazińska**

Biological properties of flavanone and its hydroxy derivatives

P54 **Kamila Agnieszka Butowska**

Functionalized silver nanoparticles interactions with model mutagen ICR-191

P55 **Agnieszka Chrustek**

Effect of pasteurization on melatonin concentration in breast milk

P56 **Monika Ciuksza**

The structural-functional analysis of complexes of plasmid replication initiation protein, Rep, and the ssDNA DUE

P57 **Monika Ćwiklińska**

Multi-compartment hydrogel capsules for topological 3D co-culture studies

P58 **Joanna Dembska**

Screening of microscopic fungi strains for the production of biosurfactants

P59 **Wojciech Dzięgielewski**

Plant histone deacetylases mutants obtained with CRISPR-Cas9

P60 **Natalia Gniada**

Antioxidant properties of plants of the genus Lycium

P61 **Maria Hayder**

The influence of gastrointestinal digestion
on metallic nanoparticles of plant origin

P62 **Mariami Jasaszwili**

Adropin stimulates ERK1/2 and AKT phosphorylation
in 3T3-L1 preadipocytes

P63 **Artur Jędrzak**

Glucose biosensor based on the hybrid microplatform
and its comparison with other testing techniques
on commercial food-samples

P64 **Aleksandra Kalińska**

Disposable tissues with silver-copper nanoparticles addition
used in dairy cows milking routine for mastitis prevention

P65 **Kacper Karczmarzyk**

Cultivation of bacterial endophytes isolated from different wheat
varieties and testing their ability to produce cellulases

P66 **Jacek Kędzierski**

Analysis of thermal stability of bacteriophages T4 and MS2

P67 **Konrad Kłosok**

Determining the population of endophytes
inhibiting *Triticum aestivum* L. 'Hondia' and *Triticum spelta* L. 'Rokosz'

P68 **Kinga Konkel**

Functionalized silver nanoparticles and their interactions
with anthracycline antibiotic - doxorubicin

P69 **Daria Kowalczykiewicz**

Immobilized enzymatic cascade
for the continuous flow trehalose production

P70 **Brygida Kruzińska**

The most frequent mastitis pathogens
occurring in small polish dairy herds

P71 **Karolina Księżarczyk**

Chemical modulation of phage stability

P72 **Joanna Laskowska**

Estimation of cytotoxicity of cinnamon oil on breast cancer cell line

P73 **Natalia Leciejewska**

Nonsteroidal selective androgen receptor modulator Enobosarm affects lipid metabolism in isolated rat adipocytes

P74 **Oskar Lipiński**

The effect of Qo site inhibitors of cytochrome bc1 on slow- and fast-relaxing forms of semiquinone generated in Qi site

P75 **Alicja Malik**

Levels of alpha amylase inhibitors and toxic fractions of gluten in sourdough bread

P76 **Paulina Marciniak**

Cheese whey permeate as a renewable substrate for polyhydroxyalkanoates synthesis

P77 **Anna Marczak**

Preliminary electrochemical study of azathioprine interaction with DNA

P78 **Paulina Markowiak**

The effect of newly elaborated synbiotic preparations on the genotoxicity of chicken faecal water

P79 **Joanna Markowicz**

a-mangostin, a natural compound from *Garcinia mangostana* Linn exerts selective toxic activity against squamous carcinoma cells through inhibition of proliferation, adhesion and apoptosis induction

P80 **Jarosław Mazur**

Role of Hrr25 kinase in the regulation of the Elongator complex

P81 **Lidia Mielcarz-Skalska**

The impact of nZVI on *Lolium westerwoldicum*

P82 **Julia Mironenka**

Multidirectional MTBE extraction

P83 **Anna Myczka**

Molecular detection of *Anaplasma* spp. in game animals

P84 **Patrycja Ogonowska**

RNA purification and selection of stable reference gene
in qPCR technique – why it matters?

P85 **Michał Pierański**

Non-antibiotic therapy for neonatal Group B *Streptococcus prophylaxis*

P86 **Adrianna Rutkowska**

Immunocytochemical analysis of the specificity of EGFRvIII binding
L8A4 antibody in tumor cell lines

P87 **Karolina Skłodowska-Jaros**

When bacteria circulate
– microfluidic application for screening of bacteria cultures

P88 **Xymena Stachurska**

Evaluation of the phage-antibiotic synergy (PAS)
based on different variables of the double-layer agar (DLA) method

P89 **Oktawian Stachurski**

Double-head cationic lipopeptides as the effective antimicrobial agents

P90 **Karolina Szacherska**

The synthesis of polyhydroxyalkanoates
by *Paracoccus homiensis* from volatile fatty acids

P91 **Magdalena Szymańska**

Immobilization of recombinant hydrolytic domain of PelA
from *Pseudomonas aeruginosa* involved in biofilm formation
on bacterial biopolymer as a carrier

P92 **Katarzyna Świerkula**

Examination of the dynamics of the *Leishmania major* pteridine
reductase 1 enzyme in complex with methotrexate

P93 **Paulina Torke**

Bacteria as microbuilders - sand bioconsolidation processes

P94 **Wojciech Wiertelak**

Analysis of interactions between nucleotide sugar transporters
using the NanoBiT system

P95 **Natalia Wiktorczyk**

The molecular characterization of *Listeria monocytogenes* strains isolated from fish and fish processing plants

P96 **Ewelina Wojda**

Determination of anti-adhesive and antimicrobial properties of *Vaccinium macrocarpon* berry extract towards *H. pylori* ATCC 43504 strain

P97 **Aleksandra Wosztyl**

Synthesis of peptide stabilised gold nanoparticles

P98 **Milena Reszka**

Dual fluorescent indicator for determination of B-glucosidase activity

P99 **Maria Kuznowicz**

Multicomponent system magnetite/lignin/polydopamine-glucose oxidase as functional biosensor for glucose detection

P100 **Sandra Kaźmierczak**

Cellulase activity in Superworm *Zophobas morio* (Coleoptera: *Tenebrionidae*)

Keynote lectures

Gretchen Edwalds-Gilbert

Dr. Gretchen Edwalds-Gilbert is currently a Fulbright Scholar at the University of Warsaw Institute for Genetics and Biotechnology; she is an Associate Professor of Biology at Claremont McKenna, Pitzer, and Scripps Colleges in California. She completed her Ph.D. at the Weill Medical College of Cornell University in New York City,
and did post-doctoral research at the University of Pittsburgh School of Medicine and the Beckman Research Institute of the City of Hope in Duarte, California. Dr. Edwalds-Gilbert is a member of the Council on Undergraduate Research (CUR) and serves as a CUR Councilor for the Biology Division. In her own molecular biology lab, she has supervised 58 undergraduates, many of whom are from groups underrepresented in science.

In her research, Dr. Edwalds-Gilbert focuses on the regulation of gene expression under stress response, and she has received funding from the Keck Foundation and the Howard Hughes Medical Institute for this work. Cells encounter stress through their interactions with the environment and either respond successfully, maintaining homeostasis, or do not, leading to cell death. Changes in gene expression critical to resolving the stress response are regulated at many stages, including gene transcription, pre-mRNA alternative processing, mRNA modification, transport, and translation. Regulation of translation after stress exposure is associated with many diseases, including cancer, and is also relevant to environmental stresses organisms face with climate changes.

Regulation of gene expression by mRNA translation in response to stress

Gretchen Edwalds-Gilbert

Fulbright Scholar, University of Warsaw Institute for Genetics and Biotechnology, Associate Professor of Biology, Claremont McKenna, Pitzer, and Scripps Colleges, Claremont, California, USA

The environment plays a critical role in the life of a cell. Extremes of temperature or water availability, for example, can place tremendous stress on a cell. Cells either respond successfully to these changes, maintaining homeostasis, or do not, leading to cell death. Various factors regulating cell growth affect a cell's ability to respond; these include gene transcription, pre-mRNA processing, mRNA modification, transport, and translation. The process of translation plays an important role in regulated steps in the gene expression pathway that permit rapid response to environmental changes. The study of translation, therefore, can provide a better understanding of how cells respond to changes in their environment, specifically to different stress conditions, and will provide new and important insights in regulation of gene expression. As cells experience existing and new forms of stress, whether through chemical exposure or climate change, the understanding of translational regulation will be essential to determining what is happening to these cells and how they respond.

Paweł Sachadyn

Paweł Sachadyn completed his PhD dissertation at the Faculty of Chemistry of the Gdańsk University of Technology in 2000. He carried out several research projects in the field of molecular biotechnology and molecular diagnostics. One of the principle directions of his work was concentrated on the application of MutS protein as a tool for the analysis of mutations and pre-mutational changes in DNA. From 2005 to 2008, as a fellow of the Foundation for Polish Science, he conducted the studies on mammalian regeneration in the Wistar Institute in Philadelphia. Further, he developed his research towards the molecular basis of mammalian regeneration with particular regard on its epigenetic aspects. From 2014 to 2018, he participated in the REGENNOVA programme on novel technologies for pharmacological stimulation of regeneration as one of project leaders. He was promoted to the rank of Associate Professor of the Gdańsk University of Technology in 2015. In 2017 he became the head of the Laboratory for Regenerative Biotechnology. Dr. Sachadyn believes that human genome contains complete information that defines the structure and functions of organisms and that silencing of developmental genes is responsible for the decline of regenerative abilities in adult mammals. This assumption led him to the hypothesis that temporary reversal of the epigenetic repression was possible to enhance healing potential. Dr. Sachadyn and his research group work to delineate novel strategies of regenerative medicine based on the pharmacological activation of endogenous regenerative potential using innovative epigenetic therapies.

Mammalian regeneration

Paweł Sachadyn

Laboratory for Regenerative Biotechnology
Gdańsk University of Technology

Regeneration is essential to all multicellular organisms, but different species display varied regenerative abilities. Certain invertebrates show almost unlimited regeneration capability. The emblematic examples to mention are the planarians which reconstitue into whole animals from isolated pieces or *Turritopsis dohrnii* known as the immortal jellyfish, which is capable of rejuvenating to its earlier developmental form of the polyp. The vertebrates do not regenerate from body fragments. The vertebrates are uncommon to restore lost organs, however, there are exceptions such as urodele amphibians that are able to re-grow limbs, regenerate transected spinal cords, and partially resected hearts. The regenerative abilities of mammals are very limited. Mammals do not re-grow lost limbs and digits. As a principle, central nervous system and hearts of mammals are not able to regenerate, and skin wounds often heal with scarring. While the extent of restorative (epimorphic) regeneration is limited, physiological regeneration is vital to normal functions of the mammalian organism. In adult mammals, epidermis, intestinal lining, and liver regenerate, and the latter one is able to regenerate following two-thirds resection.Amazing regeneration capabilities in mammals are observed in the foetal and neonatal periods. Spectacular examples are scarless skin wound healing in the foetus, perfect heart repair after partial resection in murine neonates and functional regeneration of transected spinal cord in opossum pups. The regenerative abilities decrease with development. As epigenetic mechanisms regulate cellular and tissue differentiation and developmental processes, epigenetic changes are likely to determine the decline of regenerative abilities characteristic of adult mammals. The hypothesis is supported by the established role of epigenetic regulation in stem cell pluripotency. Mammals possess high regeneration potential, active in embryos and neonates, but blocked in adults. Temporary reversal of epigenetic repression could activate the endogenous regenerative potential. Certain chemical compounds known as epigenetic inhibitors could reverse epigenetic changes, and therefore, they are potential pro-regenerative agents. Currently regenerative medicine is associated mainly with stem cells and cell-based therapies, while much pharmacological solutions are not subject to extensive research. Epigenetic inhibitors can be used as keys to activate developmentally repressed genes thus enhancing regenerative capability of mammals. The lecture presents inspiring animal phenomena of regeneration and innovative pharmacological strategies of regenerative medicine based on the use epigenetic drugs.

Radosław Zagożdżon

Dr Radosław Zagożdżon (M. D. PhD) graduated from Faculty of Medicine at Warsaw University of Medicine (WUM) in 1996. In 1998 he received medical degree (M. D. PhD) at Center for Biostructure Research WUM.

Between 2000-2005 he worked as a postdoctoral fellow and an instructor at Beth Israel Deaconess Medical Center in clinical hospital Harvard Medical School in Boston, USA. During the next years he was a postdoctroal fellow and lecturer at University College Dublin in Irleand. He was a leader of research group under the BASTION project. He also conducted research founded by National Science Centre (Poland). In 2016 he obtained degree of DSc (habilitation).

From the begining of 2017 Radosław Zagożdżon has been a head of the Departament of Immunology, Transplantation Medicine and Internal Diseases, Transplantation Institute WUM., where he also works as a physican. He is a scientific consultant in the Faculty of Bioinformatics at Institute of Biochemistry and Biophysics (Polish Academy of Science).

Dr Zagożdzon is fundamentally interested in experience oncology, tumor immunology and immunotherapy. In his research work, he is mainly focused on examining homeostasis of redox-states in tumor and immune cells, particulary Natural Killer cells. The aim of his study is to evaluate the role of the specific antioxidative enzymes systems in a growth and cells survival, tumor cells resistance to chemiotherapy and mechanism of immune evasion in cancers.

Immunotherapy with genetic engineering as the most promising approach to cancer treatment

Radosław Zagożdżon

Department of Clinical Immunology
Transplantation Institute
Medical University of Warsaw

In the recent years, immunotherapy has been introduced with the great excitement into management of numerous malignancies. Various types of anticancer immunotherapies are currently being developed worldwide. Among these, one of the most successful is an inhibition of immune checkpoints, which last year was awarded with the Nobel prize. Indeed, monoclonal antibodies blocking signal transduction from molecules negatively controlling the activation of lymphocytes have proved successful in a considerable percentage of patients suffering from cancers like malignant melanoma or lung cancer. Alternative, conceptually very different strategies are adoptive therapies with genetically modified immune effector cells, like T lymphocytes or natural killer (NK) cells. These cells can be for instance modified with synthetic molecules named 'chimeric antigen receptors (CAR'). Introduction of CAR-encoding constructs to T lymphocytes has been shown highly efficient in some forms of hematologic malignancies, and efficacy of this approach is being extensively studied in many other types of cancer. However, both types of immunotherapies are burdened with substantial shortcomings, including potentially severe side effects of the treatment. Therefore, much effort is being applied to improving the immunotherapeutic strategies. In line with this trend, our research group studies an approach combining both immunotherapeutic concepts. If successful, we can provide a new insight into development of immunotherapies against a range of cancers.

Thomas Cremer

Thomas Cremer is a Professor emeritus of Anthropology and Human Genetics at the Ludwig Maximilians University (LMU) in Munich (Germany). In the 1970s he pioneered approaches to study nuclear architecture using a laser-uv-microbeam (built with his brother, the physicist Christoph Cremer). Together with his co-workers he established 3D FISH protocols for the visualization of DNA targets from genes to entire chromosome territories (CTs) combined with the immuno-detection of epigenetic markers. In addition to 3D-studies of nuclei in fixed cells, his group also developed methods to study the dynamics of entire CTs and chromatin domains in living cells. These studies revealed a higher order chromatin organization based on non-random 4D (space-time) higher order chromatin arrangements and led to new models of nuclear organization and function. By comparison of nuclear phenotypes in a variety of species, ranging from primates and other mammals, birds, hydra, as well as to the micro- and macronucleus of a ciliate species his group has attempted to classify universally valid, species and cell type specific features of nuclear architecture in normal cell types as compared to disease correlated features in pathological cells.

Higher order chromatin organization and the interchromatin compartment - an integrated concept of the dynamics of nuclear architecture and function

Thomas Cremer

Professor emeritus of Anthropology and Human Genetics,
Ludwig Maximilians University (LMU), Munich, Germany

The compartmentalized structure of the cell nucleus has become a key issue for theunderstanding of nuclear functions. Methodological progress has now reached a state,where we witness the rise of a new research field, called the 4D nucleome. The cell nucleus provides a biological system able to play cell-type specific, evolutionary adapted 'concerts' of gene expression in the absence of a 'conductor'. During the last two decades sophisticated biochemical and microscopic methods have become available to study higher order chromatin structure and arrangements in space and time (4D) in the context of functionally relevant proteins and regulatory RNAs. Researchers have become aware that the cell nucleus is an exceedingly dynamic organelle with a 4D architecture, which allows the genetically and epigenetically controlled 4D packaging of the genome into a hierarchy of discrete chromatin structures, inseparably connected with nuclear functions. In my lecture I will point out potential roles of the interchromatin compartment (IC) as an import-export system. IC-channels start/end at nuclear pores and permeate through the peripheral layer of heterochromatin that consists of lamina associated chromatin domains (LADs). They further pervade between chromatin domains (CDs) in the nuclear interior, where they frequently expand into wider lacunae with nuclear bodies, such as splicing speckles. This channel system may provide preferential routes for imported proteins and noncoding regulatory RNAs to specific target sites and for guiding of ribonucleoprotein complexes (RNPs), which exit the nucleus through nuclear pores.

Barbara Kalinowska

Barbara Kalinowska is an experienced bioinformatician with a strong background in biophysics. She obtained her Ph.D. from the Jagiellonian Univerisity in the field of computational biology, specifically in the protein structure prediction. The research she performed during her doctoral studies resulted in several publications and was presented on international events. While the master studies, she gained also experience in the laboratory work and the research on the photodynamic therapy of cancer.

Barbara joined Ardigen at the beginning of 2017 and since then she has broadened her practical knowledge in the field of the modern genomics. She participated in several projects focused on topics like CNV detection, CRISPR/Cas9 screening or single-cell RNA-seq data analysis.

Real life bioinformatics in life science industry

Barbara Kalinowska

Ardigen Company

Ardigen provides analyses, tools and novel algorithms to life science companies which base their solutions on the most innovative technologies, particularly in the field of Next Generation Sequencing. Processing a large amount of multi-omics data and building tools supporting research requires combining expertise in bioinformatics with software engineering and data science skills. Ardigen aims to integrate knowledge from various domains to offer high-quality services in bioinformatics.

Selected real projects performed in Ardigen for global companies will be presented with the main focus on bioinformatical aspects. The examples will cover topics like CRISPR/Cas9 technology and single-cell RNA sequencing.

Egor Dzyubenko

Egor Dzyubenko studied biology, biophysics and bionanotechnology at Lomonosov's Moscow State University and received his diploma in 2011. His diploma thesis focussed on the dynamics of mitochondrial membrane potential during hyperglycaemic stress in cultivated neurons. He performed his PhD thesis at the Department of Cell Morphology and Molecular Neurobiology of the Ruhr University Bochum under the supervision of Prof. Dr. Andreas Faissner from 2012 until 2016. His thesis entitled 'Modifying synaptogenesis and functional state of neural networks *in vitro*: insights from antipsychotic treatments and extracellular matrix depletion' was awarded with the grade 'with distinction'. Subsequently, he moved to the University Hospital Essen and joined the NeuroScienceLab within the Chair of Vascular Neurology, Dementia and Ageing Research in 2017. In his research he tries to find out an answer on the three main questions:

- What is the role of astrocytes, ECM and peri-neuronal nets for neuronal plasticity and neuronal network function?

- Can we modify neuronal networks exogenously, and how can patients with neurological diseases benefit from it?

- Which are the molecular signals that lead to neurological recovery?

To address these challenging tasks, he combines animal models and cell culture techniques, bridging structural histochemical and electrophysiological methods (e.g., Multi Electrode Arrays, MEA). Within his studies, he establishes cutting-edge superresolution imaging tools (STED, SIM, PALM) and evaluates neuronal connectivity by computational approaches.

Studying neuroplasticity after stroke: how technologies pave the way for new ideas

Egor Dzyubenko

Chair of Vascular neurology, Dementia and Ageing Research, Department of Neurology, University Hospital Essen

Cerebral stroke remains one of the leading causes of death and disability in elderly patients. By virtue of the significant advances in recanalization strategies, most stroke patients outlive the acute episode. However, the limited efficacy of existing post-acute care foredooms many survivors to endure the long-term disabilities. Together with the increasing global burden of stroke, this indicates the urgent need for novel treatment approaches. Until recently, most of stroke research focused on supporting neuronal survival during the acute phase. A number of proposed drugs were moderately effective in animal studies, while many promising candidates have failed in clinical trials, including free radical scavengers and glutamate antagonists. However, the encouraging hope persists in the intrinsic mechanisms of brain repair and neuroplasticity. The development of novel medications promoting neuroplasticity during the post-acute stroke phase holds promise to overcome the limitations of existing therapies. The key to success in the search for efficient post-acute stroke therapies is the multi-dimensional view on the reorganization of nervous tissue. The assessment of neuroplasticity at meso-, micro- and macroscale can contribute to the synergistic effect in the understanding of nervous tissue remodeling after stroke. Here, the cutting-edge methods will be overviewed which help to obtain both morphological and functional readouts at different levels of brain organization. More specifically, the focus will be on the benefits of the interdisciplinary approach combining animal stroke model, superresolution imaging, network electrophysiology, functional imaging and computational modelling. Finally, the applications of such methodology will be exemplified by presenting a real-life strategy to target yet underexplored interactions between brain extracellular matrix (ECM), neurons and glial cells.

Alessio Mengoni

Alessio Mengoni obtained a PhD in Genetics from the University of Pavia (Italy) working on plant population genetics. Since more than 15 years he is interested in understanding the dynamics of symbiotic interactions between bacteria and plants, in particular from the point of view of bacterial genome evolution and biotechnology. He applies metagenomic and computational biology approaches to study the diversity, dynamics and functions of microbial communities, with special attention to host-associated microbiomes. He is co-founder of a spin-off company (EcolGene S.r.l.) devoted to environmental microbiology analyses. Alessio is professor of genetics at the University of Florence and visiting professor at the Intercollegiate Faculty of Biotechnology, University of Gdansk, Poland and at the School of Life Science, Sun Yat-sen University, Guangzhou, P.R. China.

Personal page @ https://www.bio.unifi.it/cmpro-v-p-160.html

Toward an integrated and predictive view of symbiotic nitrogen fixation

Mengoni Alessio

Department of Biology, University of Florence, Sesto Fiorentino, Italy

Rhizobia are bacteria which form symbiotic nitrogen-fixing nodules on leguminous plants,providing an important source of fixed nitrogen input into the soil ecosystem. The improvement of symbiotic nitrogen fixation is one of the major challenges facing agricultural research in order to reduce the usage of chemical nitrogen fertilizers toward the improvement of sustainable agricultural practices to deal with the increasing global human population. The exploitation of the wide range of (social) interactions rhizobia establish among themselves, with the soil and root microbiota, and with the host plant, could constitute a great advantage in the development of a new generation of highly effective rhizobia inoculants. Such optimization of existing symbioses and the possible engineering of novel synthetic symbioses will require also a comprehensive understanding of the genetics of rhizobia. The lecture will focus on multi-disciplinary approaches aimed at disclosing the still elusive biological features of rhizobia and of symbiotic nitrogen fixation. We combine evolutionary genomics, molecular genetics, synthetic biology, sociomicrobiology and predictive *in silico* modelling to focus toward an overall interpretative model of rhizobia-legume symbiotic interactions and then a rational selection of rhizobial strains and plant cultivars partnerships to maximize agricultural yield.

Industry lectures

Genomics and the personalisation of cancer care

Aleksander Sowa

Roche Polska Sp. z o.o.

The science of medicine has allowed us to make incredible advances in diagnosing and treating diseases. But the complexity of human biology is staggering. Every person is unique and in many ways, so are diseases. Yet the digital revolution in healthcare provides new ways to both collect high-quality data from each patient and connect it to data from large pools of other patients for analysis. This enables us to arrive at a deeper understanding of how to treat an individual. Only then can we see what distinguishes each of us as individuals, and translate that into personalised and thus improved care for every person.

Fulbright

Patrycja Gołąb

Polish-U.S. Fulbright Commission

The Polish-U.S. Fulbright Commission is an educational foundation, which most important task is the administration of the Fulbright Program in Poland. Besides the Commission, there is EducationUSA Advising Center, where Polish students can receive information and advice about studies in the U.S.

The Commission grants research or teaching-research scholarships, as well as scholarships for Masters and Ph.D. studies in the US. The Commission also administrates the BioLAB program for students of biological fields of study. Every year around 50 students benefits from the internship in one of four institutions in the United States (University of Virginia, UT Southwestern Medical Center, University of Chicago and Oklahoma Medical Research Foundation). The aim of the program is laboratory work and gaining of practical experience and knowledge.

Visit our website for more information: https://fulbright.edu.pl/

Sanofi

Sanofi supports patients in overcoming health challenges. We are a global biopharmaceutical company focused on human health. We prevent diseases through vaccines and provide innovative treatments. We support both those who suffer from rare diseases and those who suffer from chronic diseases.

Thanks to the involvement of over 100,000 employees in 100 countries, Sanofi transforms scientific innovations into healthcare available around the world. One of the 112 Sanofi factories is located in Rzeszów. Sanofi in Poland hires nearly 1,000 employees. According to data from IMS Health Polska, Sanofi is one of the leading companies in the ranking of pharmaceutical industry.

During the lecture you will gain information about Sanofi Internship Program – "Student on Board" which enables young talents to get their first professional experience.

Sartorius

As research enables humanity to defend themselves against infection and to cure diseases, modern and innovative solutions are required for drug/vaccine discovery, development and production. This is where we, as a leading international partner of biopharmaceutical research and industry come in.

Our Lab Products and services division focuses on drug discovery and development, supplying high quality laboratory instruments, consumables and excellent services to boost productivity and enable breakthroughs.

Our Bioprocess solutions division provides biopharmaceutical producers with state of the art upstream and downstream single use technologies to ensure high quality drug production.

Simply our mission at Sartorius is to empower scientists and engineers to simplify and accelerate progress in life science and bioprocessing, enabling the development of new and improved therapies and economical medication.

sartorius

Labnatek

Grzegorz Kaszyński

Labnatek is a sales distributor of hi-tech devices from companies such as Nanolive SA and CELLINIK used in nanotechnology, biotechnology, advanced microscopy and 3D bioprinting. During the industry lecture Labnatek will present two innovative research systems: BioX Bio 3D Printer (CELLNIK) and Nanolive 3D Cell Explorer (Nanolive SA).

BioX is a bioprinter used for forming a complex 3-dimensional structures such extra-cellular scaffolds or tissue-like structures created from cells suspended in a hydrogel. BioX is an independent unit, ideal for a small laboratory or as a convenient tool under every laminar flow cabinet.

Nanolive 3D cell Explorer utilizes an innovative technique of holography which enables real time non-invasive imaging of separate components of tissue and distinguishing different types of cells.

Workshops

WE and THE OTHERS - Considerations on the evolutionary roots of a peaceful or aggressive human behavior

Thomas Cremer

Professor emeritus of Anthropology and Human Genetics,
Ludwig Maximilians University (LMU), Munich, Germany

The world today is in a state of global apartheid. The rapidly growing world population, environmental pollution, climate change, social and political conflicts require new strategies of global action and coexistence to master these challenges. As a consequence of the rather 'short-sighted' Darwinian evolution, common far-sighted actions for the benefit of future generations are very difficult to plan and even more difficult to execute. The insights of evolutionary biology, however, support neither the overly pessimistic judgment that any peaceful behavior would reflect only a thin, culturally mediated crust concealing our real nature, which seems preprogrammed to violence, nor the overly optimistic expectation of an inherently peaceful nature of human kind. In this presentation I consider the evolutionary roots of a peaceful or violent human behavior. For this purpose, a look beyond our human species to other species with a social behavior is helpful.

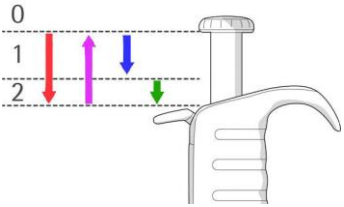
The evolution of genetic predispositions for intragroup cooperation and outgroup aggression in all social species was influenced by complex interactions of already existing genetic and epigenetic networks with environmental factors. For humans the affiliation with groups that have a significant influence on the behavior of their members, as well as the dominant culture of a society at large, have a decisive, but still little understood impact on human behavior. Some brain researchers believe that the assumption of any person's free will is an illusion. We may believe that we are doing something voluntarily, but that is just our imagination. If this would be true, there would be no individual freedom to choose between alternative courses of action, no moral self-responsibility for the consequences of our actions as is, for example, the case in a colony of ants. And indeed, our freedom to choose is often restricted. An indissoluble difficulty lies in the fact that our freedom of choice is restricted by numerous unknown constraints that are effective through the neural networks of our brains. We do not enjoy the gift of a brain created by an intelligent designer with the intention of giving us an overwhelming freedom of choice. But despite all reservations, I plead for the possibility of free decisions and thus for the possibility of self-responsible moral action. Whether this hope is true or rather an illusion, cannot be decided by experiments in neurobiological laboratories or by profound philosophical texts or by a religious dogma. This hope may be confirmed or extinguished in the actual realization of our individual and social life. At this point I do not see compelling scientific reasons to abandon this hope and want to hold on to it as an expression of my individual freedom. However, when confronted with feelings of hate, flaming up in times of crisis, an individual decision for a moral position, which runs counter the views of one's own dominant group, requires a commitment and civil courage to an extent, which we can barely imagine in times without such challenges.

Pipetting Academy - Basics of pipetting

Technique is key for pipetting: With the right technique you get the most out of your precious samples and make the best use of your valuable time while pipetting without any pain. The Sartorius pipetting academy will give you the background knowledge and practical insights to work efficiently and safely – for best results even in long pipetting series.

Module basic of pipetting shows:

- how to choose the right equipment
- the basic pipetting techniques
- the best practices in lab ergonomics
- daily maintenance and checking of pipettes



Running startups

Biolumo

Based on 3 years experience as a co-founder of a MedTech startup – Biolumo I will share how to start your own Biotech/MedTech startup and present which skills need to be learnt to have the best chances to succeed in the startup world. You will learn which factors are crucial when choosing co-founders, how to make and use the business model canvas to test your hypothesis, and also how to approach and pitch to investors or potential partners.

Presentation skills

Mikołaj Fedorowicz

Science Advocates Association

Presentation of results is part of our job, and that's a fact. For some of us its the most horrifying part others - love it. No matter are you a lover or a hater, you probably asking yourself one question: *"What can I do to get people to listen to me?"*. Not just hear but listen. I know what you thinking, and no. There will be no miracle answers, just sharing some experience. Come for some tips or bring your own to the table!

Mikołaj Fedorowicz, biotechnologist, Ph.D. student in Institute of Biochemistry and Biophysics PAS and science communicator. He started his science communication journey some time ago, in such institutions as the School of Science Festival or Copernicus Science Center. More recently he achieved second prize in FameLab Poland competition, joined the Association of Science Advocates and became co-chair of the March for Science Poland. Communication for him is pure pleasure because he loves to talk. Often too much and too loud. In a time when he does not talk, he scuba dives, dance rock'n'roll and try to juggle.

Oral presentations

Effects of Reduced Graphene Oxides on Apoptosis and Cell Cycle of Glioblastoma Multiforme

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Graphene (GN) and its derivatives (rGOs) show anticancer properties in glioblastoma multiforme (GBM) cells in vitro and in tumors in vivo. We compared the anti-tumor effects of rGOs with different oxygen contents with those of GN, and determined the characteristics of rGOs useful in anti-glioblastoma therapy using the U87 glioblastoma line. GN/ExF, rGO/Term, rGO/ATS, and rGO/TUD were structurally analysed via transmission electron microscopy, Raman spectroscopy, FTIR, and AFM. Zeta potential, oxygen content, and electrical resistance were determined. We analyzed the viability, metabolic activity, apoptosis, mitochondrial membrane potential, and cell cycle. Caspase- and mitochondrial-dependent apoptotic pathways were investigated by analyzing gene expression. rGO/TUD induced the greatest decrease in the metabolic activity of U87 cells. rGO/Term induced the highest level of apoptosis compared with that induced by GN/ExF. rGO/ATS induced a greater decrease in mitochondrial membrane potential than GN/ExF. No significant changes were observed in the cytometric study of the cell cycle. The effectiveness of these graphene derivatives was related to the presence of oxygen-containing functional groups and electron clouds. Their cytotoxicity mechanism may involve electron clouds, which are smaller in rGOs, decreasing their cytotoxic effect. Overall, cytotoxic activity involved depolarization of the mitochondrial membrane potential and the induction of apoptosis in U87 glioblastoma cells.

Regulatory effect of graphene oxide on secondary metabolism in streptomycetes

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Engineered nanomaterials are applied in many fields of everyday life. They are present in industry, agriculture and medicine. Even though there are thousands of novel nanostructures, their activity on microorganisms remains unexplored. Many of these materials were investigated in terms of toxicity, although these studies have not answered what happens with the secondary metabolism in cells (or populations) subsequently to exposition to such xenobiotics. This research aimed in studying the interaction between graphene oxide in two forms and selected bacteria from genus *Streptomyces*.

Studies were conducted on five streptomycetes including four wild strains and *S. griseus* ATCC®10137™ and reference microorganisms - *Escherichia coli* K-12 (C600), *Staphylococcus aureus* FRI 137, *Pseudomonas aeruginosa* ATCC®27583™ and *Candida albicans* ATCC®10231™. Graphene oxide was used in its unmodified form and one that was functionalised with cobalt nanoparticles. Standard microbiological methods were applied together with HPLC chromatography combined with mass spectrometry, on solid and liquid cultures. The reference microorganisms were used in the determination of antagonistic properties of streptomycetes subjected to nanostructures.

Selected nanomaterials influenced morphology, growth and metabolic activity of selected streptomycetes. One strain was stimulated to produce an antifungal compound in liquid culture. Gathered results open a novel route in research on activating streptomycetes to produce useful secondary metabolites in liquid cultures. This outcome can have importance in the further application of graphene oxide in the biotechnological production of secondary metabolites.

Preliminary study on the usability of biodegradable ZnO nanoparticles doped with Fe for iron supplementation

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Previous studies showed that ZnO nanoparticles transfer efficiently from gastro-intestinal tract and are distributed to majority of tissues and organs following their alimentary application. This coupled with biodegradability prompted us to consider them for application as drugs and functional delivery systems. The aim of this preliminary study was to validation of hypothesis that biodegradable ZnO nanoparticles maybe use as an iron delivery system to the organism. Secondary, aim was to evaluate the influence of iron supplemented with the nanoparticles carriers on iron levels in the tissues. ZnO:Fe nanoparticles were administrated via intra gastric (IG) route suspended in RO water (10 mg/kg; 0.3 ml/mouse) to Balb-c mice (n=4). Simultaneously, mice received 0.3 ml RO water IG. After 24h mice were sacrificed and organs were collected for analyses. All collected tissues were evaluated by Atomic Absorption Spectroscopy (AAS) and organs crucial for iron storage and metabolism (liver, spleen and brain) were subjected to the Pearl's staining to visualised the iron aggregates for in-tissue cytometry. AAS results showed significant increase of iron content in liver and brain, and a trend for small intestine, spleen and a heart. On the other hand the in-tissue cytometry results of iron aggregates in the liver and spleen. In the brain significant increased of iron content was not reflected by Pears staining, most probable due to the different mechanism iron storage (diffused accumulation in the oligodendrocytes). Reassuming, biodegradable ZnO nanoparticles proved valid of the iron supplementation. Secondly, as our preliminary results showed increased accumulation of iron aggregation in liver and spleen, further research is needed to evaluate impact of a supplementation on the iron homeostasis.

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Nanomaterials based on bacterial cellulose with increased water absorption

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Bacterial cellulose, also called nanobiocellulose due to the size of its fibers, is a biomaterial produced by bacteria, mostly *Komagataeibacter xylinus*, with many beneficial and unique properties. Its porosity, biocompatibility, high chemical purity, non-toxicity and simple manufacturing process allowed to use this material in number of industries and fields of science. To further improve properties of bacterial cellulose for a specific purpose, for example to give it antimicrobial properties, many modifications can be also easily applied, both at *in situ* and *ex situ* level.

The aim of the present research was to obtain bacterial cellulose based bionanomaterial with improved absorption properties by *ex situ* chemical modification of its structure.

Bacterial cellulose pellicles were obtained from *Komagataeibacter xylinus* cultures conducted for 7 days under stationary conditions. After purification in a solution of sodium hydroxide and neutralization with large amount of distilled water, BC pellicles were immersed in citric acid with the addition of different catalysts and incubated for 24 h at 28°C and then for 1.5 h at 160°C. The current study showed, that obtained bacterial cellulose based biopolymer differed in structure from standard cellulose. It was revealed, that modified bacterial cellulose was characterized by several times higher water swelling properties as well as water holding capacity. Furthermore, the thickness of the obtained biomaterial after citric acid modification, significantly increased compared to unmodified cellulose, which resulted from preservation of the three-dimensional structure of bacterial cellulose during the drying process.

Fullerenes triggered G2/M-phase arrest of liver cancer cells by mechanotransduction towards membrane proteins, cytoskeleton and the nucleus

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Introduction and objective: Degradation of the extracellular matrix (ECM) changes the physicochemical properties and dysregulates ECM-cell interactions, leading to several pathological conditions, such as invasive cancer. Carbon nanofilm, as a biocompatible, easy to functionalise and nano-sized material, could be used to mimic ECM structures, changing cancer cell behaviour to perform like normal cells.

Methods: Experiments were performed *in vitro* with HS-5 cells (as a control) and HepG2 and C3A cancer cells. An aqueous solution of fullerene C_{60} was used to form a nanofilm. The morphological properties of cells cultivated on C_{60} nanofilms were evaluated with light, confocal, electron and atomic force microscopy. Both cell viability and proliferation were assessed by XTT and BrdU assays. Immunoblotting and flow cytometry were used to evaluate the expression level of proliferating cell nuclear antigen and determine the number (percentage) of cells in the G2/M phase.

Results: All cell lines were spread on C_{60} nanofilms, showing a high affinity to the nanofilm surface. We found that C_{60} nanofilms mimicked the niche of cells, was biocompatible and non-toxic, but the mechanical signal from C_{60} nanofilm created an environment that affected the cell cycle, which lead to reduced proliferation.

Conclusion: In studies on liver cancer cells, we documented that the signal derived from the fullerene nanofilm is preferentially chosen by the cell, creating an environment conducive to adhesion and colonisation. Furthermore, cells settled in this way decreased the ability to form spheroids, caused the cell cycle arrest in the G2/M phase and decreased proliferation. It can be expected that the incorporation of fullerenes in the ECM of liver cancer cells can reduce cell malignancy and improve tumour therapy.

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The regulation of AP2-α transcription factor via WWOX protein and its influence in bladder carcinogenesis

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WWOX protein interacts with several transcription factors including AP2-α and AP2-γ, which belong to AP2 family. Literature data indicate that in cytoplasm WWOX inhibits AP2 transcriptional ability and therefore stops an activation of downstream target genes. In majority, researchers claim that AP2-γ acts as an oncogene, thus WWOX represses the pathways associated with this protein by inhibiting its transfer from the cytoplasm to the nucleus. However, molecular mechanism of interaction between WWOX and AP2-α is still unclear. Our preliminary results of bioinformatics shows that bladder cancer patients with high *WWOX* and *TFAP2A* demonstrate better prognosis compared to those with lower *WWOX*.

The purpose of present experiment was to examine diverse properties of the RT-112 bladder cancer cell line (grade 2) with high *TFAP2A* and various levels of *WWOX* (silenced or overexpressed). Looking at created *in vitro* model, we noticed metalloproteinase *MMP9* expression decreased in case of high *WWOX* (these are cells which do not pass through the basal membrane) and also reduced programmed cell death event. As far as cell proliferation, viability and mitochondrial potential are concerned, no statistically significant differences were observed compared to the cells with silenced *WWOX*. Moreover, reduction of caspase positive/death cells after induction via staurosporin was revealed in this model. Simultaneously, the level of *WWOX* does not affect adhesion to ECM proteins or clonogenicity (colony formation assay).

Based on the above results, we can speculate that the level of *WWOX* expression and participation of AP2-α; factor, has different effects on the characteristics and properties of low grade bladder cancer.

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Identification of molecular markers for detection and characterisation of single breast cancer circulating tumour cells in different epithelial-to-mesenchymal states

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Circulating tumour cells (CTCs) are metastatic precursors shed by primary tumour into blood, what makes them a valuable source of information about primary and metastatic lesions. To better understand steps of metastatic cascade it is important to molecularly profile these rare and heterogenous CTCs on a single cell level. Recent studies indicate that evasion of the immune system might be related to different phenotypical state of cancer cells undergoing epithelial-to-mesenchymal transition (EMT) by disrupting the expression of immunoproteasome genes.

In this study, we aim to analyse expression of genes in single cells connected with EMT and subunits of immunoproteasome.

To obtain single cells of different EMT state, two cell lines were used - MCF7 and MDA-MB-231. In order to simulate CTC isolation from clinical samples, cells from cell lines were spiked into healthy donors blood samples. CTCs were enriched by density gradient centrifugation, followed by immunofluorescent staining for epithelial and mesenchymal markers and removal of CD45-positive cells with immunomagnetic beads. Single positive cells were isolated with a micromanipulator, processed according to a modified Smart-Seq2 protocol, which provided a template to analyse expression of genes related to EMT phenotype (*CK19*, *EpCAM*, *CDH1*, *VIM*, *PLS3*, *SERPINE1*) and immunoproteasome (*PSMB8*, *PSMB9*, *PSMB10*) by qPCR.

Statistically significant differences in expression of E*pCAM, CDH1, VIM, PLS3* and *SERPINE1* were noted between cell lines, what allowed to discriminate their epithelial and mesenchymal state. *PSMB8* expression was increased in mesenchymal, in comparison to epithelial cell line.

In conclusion, significant differences in expression of epithelial and mesenchymal markers are observed after single CTCs isolation, enabling classification to EMT classes. Lack of differences in most genes linked with immunoproteasome requires searching for alternative causes of increased aggressiveness of mesenchymal cells.

Rs10877012 polymorphism in *CYP27B1* gene in women with diagnosed breast cancer: preliminary study

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Since 2.1 million cases of breast cancer are diagnosed every year and it causes the most deaths from all cancers in women, there is an urgent need to define more precisely what factors may play role in the etiopathogenesis of the disease. Dysregulation of the metabolic pathway of vitamin D is a characteristic feature of breast lesions and the level of the enzyme of CYP27B1 is reduced alongside with the progressive development of the cancer. Due to the hydroxylation at the first carbon atom catabolised by CYP27B1 enzyme, vitamin D becomes antiproliferative, prodifferentatative and proapoptotic agent.

The aim of this study was to determine the association between CYP27B1 (rs10877012) polymorphism and the occurrence of breast cancer.

The frequency of the T- and G-alleles at the rs10877012 polymorphic site in CYP27B1 gene was compared in 78 women with breast cancer confirmed histopathologically and 103 healthy control women. DNA was isolated from peripheral blood cells and genotyped using PCR-RFLP method. Statistical analyses were performed using GraphPad Prism software with P-value $p \leq 0.01$ considered as significant. Pearson's chi-square test was used to analyse the allele frequencies for Hardy-Weinberg equilibrium (HWE). Allele frequencies were compared using the Fisher's test.

Among women with cancer, the genotype TT (47%) was found more frequently than in healthy women (29%). The prevalence of the T-allele in the study group was 0.63, compared to the control group - 0.46. The obtained results indicate a correlation between the presence of the GG genotype and decreased chance of breast cancer (OR = 3.01; 95% CI: 1.41-6.40, P =0.004). Therefore, the presence of the T-allele at the rs10877012 polymorphic site may be an important element associated with the incidence of breast cancer. Moreover, the data suggest the importance of vitamin D metabolism in breast cancer cells and the great impact of genetic variations on the key enzymes of vitamin D pathway.

Heavy metal tolerance in bacteria isolated from soil contaminated with polycyclic aromatic hydrocarbons (PAHs)

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Anthropogenic pressure on the environment can result in soil contamination with polycyclic aromatic hydrocarbons (PAHs) and heavy metals. PAHs are chemical compounds which include several benzene rings arranged in various combinations. PAHs are strongly mutagenic, carcinogenic and teratogenic, therefore they are dangerous for both humans and environment. PAHs contamination is frequently related with accumulation of heavy metals in soil. Furthermore, industrial areas, where petroleum and metals are exploited, have a high risk for further contamination. One of the most effective methods for the removal of chemical pollution is bioremediation. Among solutions that mitigate this problem, the use of autochthonic bacteria seems to be the most promising because of its ecological meaning and inexpensiveness. The aim of this study was to isolate autochthonic bacteria from petroleum-contaminated soil and test their survival in medium contaminated with polycyclic aromatic hydrocarbons and tolerance to heavy metals.

In this research bacteria where isolated from soil which was collected from area contaminated with following PAHs: naphthalene, anthracene, chrysene, fluoranthene, phenanthrene and phenol. Bacteria were incubated on Mineral Medium (MM) enriched with high PAH concentration. Afterwards, 21 strains, that maintained their viability in the contaminated medium, were selected and subjected to medium contaminated (0,195 mM - 50 mM) with heavy metals, such as Zn, Cu, Co, Ni, Cd, Hg, Pb.

Isolates showed a diversified response to the studied selective conditions. None of the strains demonstrated tolerance to all of heavy metals, nor to all of the used concentrations. However, some strains could grow in the presence of few heavy metals. Obtained results show that contaminated soil contains autochthonic bacteria that were environmentally selected to resist the contamination. In further steps their biotechnological potential to degrade petroleum substances will be studied.

The study of the accumulation of insecticides and the impact on cell permeability in entomopathogenic fungus *Beauveria bassiana*

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In this studies, the accumulation of pyrethroid insecticides as well as their effect on the cell wall and membrane permeability of *Beauveria bassiana* was examined.

Three pyrethroids insecticides were used in the research: λ-cyhalothrin, α-cypermethrin and deltamethrin in concentration of 5 mg/L.

In order to determine the accumulation of these compounds in the biomass of entomopathogenic fungi, 5-day old cultures were extracted with ethyl acetate and methylene chloride and then the content of insecticides in *B. bassiana* mycelium by gas chromatography coupled with mass spectrometry (GC-MS) was determined.

The content of insecticides in the culture medium as well as in the *B. bassiana* mycelium were compared. The obtained results indicate that this compounds accumulated in tested fungus mycelium (λ-cyhalothrin 71.86%, α-cypermethrin 70.16% and deltamethrin 99.6%) .

To determine the effect on the cell wall and membrane permeability, biomass from 48-hour cultures was subjected to mineralization using nitric acid. The content of magnesium and sodium in the samples was determined by atomic absorption spectrometry (AAS) using a Spectra 240 FS apparatus.

B. bassiana cultivation with α-cypermethrin and deltamethrin had the highest influence on the cell wall and membrane permeability. It caused a decrease in magnesium content by 69.40% and 70.88% respectively, and a decrease in sodium content by 78.69% and 89.09% respectively (in comparison to the biotic control).

In conclusion, pyrethroid insecticides penetrate from the culture medium to *B. bassiana* mycelium and have an adverse effect on the permeability of the cell membranes of this microorganism.

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Investigation of diversity of bacterial microflora in the stratosphere

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The stratospheric microbiome has been investigated many times using the methods of classical microbiology. In this research a few modern methods, including NGS sequencing and multiple displacement amplification of DNA, were in use. The analysis of metagenome helped to determine the content of various species of bacteria in the sample collected in the stratosphere by a hydrogen-filled balloon holding a gondola with specialistic apparature. Also the comparison of DNA amounts in specimens collected in the stratosphere and in a ventilated room within a building has been done, which has shown that the stratospheric air is not poor in bacteria, unlike the previous notions suggested. By running experiments with a vacuum chamber, the possibility of destruction of bacterial cells due to a pressure shock during the fall of the gondola with the samples was disproven. Collected cells were unable to be cultivated on agar media in any temperature. The results of the metagenomic 16S rRNA coding gene sequencing revealed that a great amount of the collected DNA came from undescribed species. The most dominant geni among the known bacteria are *Enterococci*, *Staphylococci* and *Bacilli*.

Analysis of antibiotic resistance of *Staphylococcus* spp. strains isolated from pigs

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Swine are potential carriers of *Staphylococcus* spp., which can cause various health conditions, such as arthritis or porcine ear necrotic syndrome (PNES), thus increasing the cost of treating the entire herd. Due to the possibility of transmission of pathogenic strains from pigs to humans, identification of potentially pathogenic staphylococci and their drug resistance have epidemiological and epizootiological significance. The aim of this study was to determine the antibiotic resistance of *Staphylococcus* spp. isolated from healthy and infected pigs.

The research material consisted of 23 strains of staphylococci isolated from 10 out of 22 pigs. Swabs were taken from the nasal cavities, the outer ears, the vaginae, post mortem from the joints affected by inflammation, and the lung tissue of individuals diagnosed with fibrous polyserositis. Restriction fragment length polymorphism - based polymerase chain reaction (PCR-RFLP) analysis of the *gap* gene using the enzyme *Alu*I was used for species identification. Antimicrobial susceptibility testing was performed by disc diffusion method recommended by EUCAST.

Out of 23 isolates, 11 were classified as *S. aureus*, three isolates as *S. caprae* / *S. saprophyticus*, two isolates as *S. arlettae*, *S. fleuretti*, *S. hyicus* and *S. sciuri* and one isolate as *S. pasteuri*. The analysis of antibiotic resistance revealed the presence of four methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS) strains (three *S. caprae* / *S. saprophyticus* and one *S. arlettae*), as well as two methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Among analysed staphylococci, 16 strains (70%) were multidrug resistant (MDR). In four (17%) isolated strains, a constitutive mechanism of macrolide-lincosamide-streptogramin B (MLS_B) resistance was noted.

Based on the research, it was found that both healthy and infected pigs can be carriers of MRSA strains and multidrug resistant staphylococci belonging to different species.

Anti-lysine DNA aptamer — a novel tool for purification of recombinant proteins

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Affinity chromatography is a method used to purify different types of molecules. It is based on the specific interaction between a ligand, immobilized on a solid support, and an analyte e.g. a protein present in the solution. Nucleic acid aptamers emerge as new solutions for affinity chromatography. Aptamers are short single stranded RNA or DNA oligonucleotides. Due to their secondary and tertiary structures, they can bind other molecules including peptides, proteins and whole cells. Positively charged poly-lysyl peptides enhance cell or tissue adherence to surfaces such as plastic or glass. Moreover, lysyl oligopeptides can be used to increase recombinant protein solubility. Up to now an affinity chromatography system for purification of proteins tagged with a lysyl oligopeptide was not available. Therefore, the aim of the study was to develop an affinity chromatography system based on an anti-lysyl DNA aptamer which could be used for purification of recombinant proteins tagged with a lysyl-tag. The selection of DNA aptamers was performed using the single bead SELEX method. After the sixth round of selection, the pool of ssDNA molecules was cloned and sequenced. Specificities of selected aptamers were analyzed using quantitative real-time PCR. Among the tested sequences, the B7 aptamer was characterized by best properties in terms of binding to the lysyl-tag. The affinity chromatography system using the B7 aptamer and the lysyl-tag was optimized and characterized. Possible applications of the aptamer are the purification of lysyl-tagged recombinant proteins and ELONA test.

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Structural pre-adaptation and episodic selection drove evolution of moonlighting activity of Hsp70 co-chaperone Zuotin

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Proteins are dynamic molecules, able to both gain and lose activities in the course of evolution. Yet, the mechanisms involved are poorly understood. Here we track the evolution of a novel moonlighting activity of *Saccharomyces cerevisiae* Zuotin. Zuotin, a multi-domain eukaryotic Hsp70 co-chaperone, is primarily ribosome-associated, involved in folding of nascent polypeptide chains. In plants, protists and animals, the C-terminal SANT domains, which were lost at the base of the fungal lineage, play off- ribosome regulatory roles. The C-terminus of the shorter fungal Zuotin also plays a moonlighting regulatory role in *S. cerevisiae*, activating Pdr1, a transcription factor presents only in the *Saccharomycetaceae* clade. The C-terminal domain of fungal Zuotin is a 4-helix bundle (4HB), with a short C-terminal hydrophobic segment folding back into the bundle, forming a “plug” stabilizing the structure. Hydrophobic residues of this segment are key to Pdr1 activation. To trace the evolution of this function, we first solved the structure of the 4HB region of human Zuotin. This segment also forms a 4-helix bundle, but it does not contain a plug. Subsequent analysis of animal and fungal Zuotin sequences revealed that the plug likely evolved as a result of genomic rearrangement upon SANT domain loss. In the *Saccharomyces*lineage, the 4HB was subjected to episodic positive selection and the plug became particularly hydrophobic, a feature adopted later for its novel regulatory role. Thus, Zuotin evolved off-ribosome functions twice - once involving SANT domains, then later in fungi, after SANT domain loss, by coopting the hydrophobic plug.

Microfluidic screening of antibiotic susceptibility at the single-cell level

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Antibiotic resistance is an important threat to the public health. Level of antibiotic resistance of bacteria is measured as the minimum inhibitory concentration (MIC), which is the lowest concentration of a drug that prevents visible growth of bacteria. The inoculum effect describes a dependency between the MIC of an antibiotic and the concentration of bacteria in the sample: the less the bacteria, the lower the MIC. In the extreme case of a population that contains only a single bacterium, MIC is known as single-cell MIC (scMIC). scMIC is important from the perspective of public health, as the presence of antibiotic at concentration of scMIC in a large population of bacteria drives the evolutionary pressure towards strains resistant to this antibiotic. However, efficient assessment of scMIC values for large numbers of experiments has not yet been shown.

Here (1), we demonstrate a method of determining scMIC values in hundreds of replications per experimental run. We generate a series of emulsions of different concentration of antibiotic at a step emulsifier (2). Each emulsion is separated from the others by being encapsulated in a third immiscible phase, and transferred to a piece of tubing, where all the separated tankers can be incubated to provide for growth of bacteria. We show that we were able to measure scMIC value of cefotaxime in E. coli in hundreds of replications, also recording the inoculum effect.

The method described in this study is a milestone in the field of antibiotic resistance at a single-cell level. The method of screening multiple chemical conditions in emulsions of subnanoliter droplets without labeling can be also deployed in other fields of research, wherever several reaction conditions should be replicated hundreds or thousands of times.

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Potential removal of cytostatic pharmaceuticals by *Tritirachium album* - sorption study

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Growth in the use of anticancer drugs leads to increased levels of cytostatics released to the environment. They occur in surface, ground and even drinking water. The concentration of them in the aquatic environment depends on the location, matrix and specific drug, however, their concentrations in hospital effluent comes up to a quarter million ng/L. These compounds are recalcitrant in natural waters and they are not effectively removed during wastewater treatment processes, what means that they could be potentially risky to the ecosystems. Because of that researches about the effective elimination of cytostatic drugs from environment requires intensive research. Wastewater fungal treatment seems to be a promising alternative to solve this problem.

The aim of this work was to establish sorption ability of fungal strain *Tritirachium album* to eliminate two selected anticancer drugs: bleomycin and vincristine. Species identification was based on the Internal Transcribed Spacer ribosomal DNA sequence analysis. Sequences were compared to the NCBI GenBank-deposited sequences using BLASTn algorithm. Sorption test was conducted by placing 0.1 g of dead (autoclaved) fungal biomass in phials containing 10 ml of cytostatics aqueous solutions in concentration 10 mg/L. The process was carried out by 4 hours and the loss was measured at regular intervals using spectrophotometer UV-Vis. Samples were tested in triplicates.

Results obtained in this test are useful in determining the role of physical drug elimination. They show that removal of chosen cytostatic differ significantly and depend on drug. The greater elimination (at level of 30%) show bleomycin. Vincristine loss was smaller and after 4 hours it was about 20%. In case of both tested anticancer drugs sorption capacity was saturated after 2 hours.

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The interaction of Hsp70/Hsc20 chaperones with folded protein substrate

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Hsp70 molecular chaperones and their J-protein co-chaperones have critical roles in protein folding, transport, disassembly of complexes and biogenesis of cellular structures. A single Hsp70 can interact with many J-proteins, which stimulate the ATPase activity of Hsp70 and mediate the binding of specific substrate, but so far, most experimental studies on Hsp70-substrate interaction have been performed in vitro using short peptides. Because of that, the mechanism of Hsp70/J-protein interaction with completely folded substrates, which is frequent in the cell, is poorly understood.

To investigate the productive Hsp70/J-protein/substrate interaction, we used the highly specialised Hp70(Ssq1)/Hsc20 system from *S. cerevisiae*, which is critical in Fe/S cluster biogenesis and has a natively folded protein - Isu - as substrate. We were able to pufify a stable Ssq1-Hsc20-Isu complex in amounts sufficient for biochemical analyses, thanks to the Ssq1 T239A variant stabilizing the ATP-bound state. Simultaneously, we combined molecular docking and molecular dynamics simulations to predict the structure of the triple complex. To verify this model, we applied hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS), comparing deuteration rates between separate proteins, double Hsp70(Ssq1)-substrate complex and the tripartite complex. The differential deuteration patterns suggest that the J-domain of Hsc20 binds Hsp70 at the interface formed by the ATPase domain, substrate-binding domain and the interdomain linker, consistently with our structural model. Moreover, the deuteration patterns on sites of interaction with Isu on both Hsp70(Ssq1) and Hsc20 also support our model, suggesting the initial interface between ATP-bound Hsp70 and protein substrate. This, together with our previous results, indicates that the interplay with J-domain partner is of particular importance for binding native protein substrates.

Yeast-model-based study identified myosin- and calcium-dependent calmodulin signalling as a potential target for drug intervention in chorea-acanthocytosis

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Chorea-acanthocytosis (ChAc) is a rare neurodegenerative disease associated with mutations in the *VPS13A* gene, one of the four *VPS13* homologues present in human. ChAc is characterised by complex neurological signs such as chorea; dystonia and twitches. Currently, there is no effective therapy and treatment is limited to palliative care, due to unknown molecular pathology of ChAc and poorly defined function of VPS13 proteins. To investigate the role of VPS13 proteins, we use yeast *Saccharomyces cerevisiae*, in which one *VPS13* gene is present, as a model organism. Yeast Vps13, like human VPS13A, is involved in vesicular protein transport, actin cytoskeleton organisation, phospholipid metabolism and localises at membrane contact sites.

In this study, we performed a genetic screen for multicopy suppressors of the *vps13*Δ mutation. A fragment of the *MYO3* gene, encoding Myo3-N (the N-terminal part of myosin, a protein involved in endocytosis), was isolated. Myo3-N protein contains a motor head domain and a linker with IQ motifs which mediate the binding of calmodulin - a central cellular calcium sensor but also a negative regulator of myosin function. The amino acid substitutions that disrupt the interaction of Myo3-N with calmodulin resulted in the loss of *vps13*Δ suppression. This pointed to calcium signalling as a pathway important for the suppression. We showed that production of Myo3-N downregulates the activity of calcineurin, a protein phosphatase regulated by calmodulin. Moreover, drugs which interfere with calcium signalling also acted as suppressors. These results show that defects associated with *vps13*Δ; could be overcome, and point to a functional connection between Vps13 and calcium signalling as a possible target for chemical intervention in ChAc.

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The transcription factor TCF7L2 confers and maintains postmitotic neuronal identities in vertebrate thalamus and habenula

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Background The thalamus and habenula are essential hubs for sensory information, adaptive task control, and reward processing. We still do not fully understand the molecular mechanisms that govern the postmitotic differentiation of these structures. Such knowledge is required to elucidate the aetiology of thalamic and habenular dysfunctions that are seen in multiple neuropsychiatric disorders. The TCF7L2 transcription factor is specifically induced in thalamo-habenular region during neurogenesis, and its expression continues undiminished in adult neurons. We used two mouse models to investigate the role of TCF7L2 in the acquisition and postnatal maintenance of postmitotic identities of neurons in the thalamus and habenula.

Results In embryos with the total knockout of *Tcf7l2*, neurons did not segregate to form separate nuclei, and anatomical outer boundaries of the thalamus and habenula were blurred. Thalamic and habenular neurons did not grow axons toward their targets, and their afferent connections were disorganized. It was associated with downregulation of cell adhesion and axon guidance genes. The expression of the pan-thalamic and pan-habenular selectors *Gbx2* and *Pou4f1*, respectively, despite early induction during neurogenesis was lost at later development. Sub-regional patterning by other transcription factor genes was also abolished, but generic glutamatergic neurotransmitter identity was properly established and maintained. In contrast, the post-developmental knockout of *Tcf7l2* only mildly affected the patterning of the thalamus. Nevertheless, numerous region-specific synaptic transmission genes were downregulated in the thalamo-habenular domain.

Conclusion TCF7L2 orchestrates a network of transcription factor genes to regulate postmitotic molecular differentiation, segregation of neurons and axon path-finding in the thalamo-habenular domain. Continuous expression of TCF7L2 in adult is required to maintain terminal differentiation programs in this region.

Analysis of HvTIP1;1 gene expression coding for aquaporin in barley (*Hordeum vulgare* L.) after treatment of seedlings with compounds inducing oxidative stress

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Aquaporins are a group of membrane proteins that participate in many physiological processes of plants, including the response to a abiotic stress, including an oxidative stress. The tonoplast intrinsic protein (TIP) subfamily often takes part in this kind of response because it is responsible for the maintenance of cell turgidity and the osmotic balance between vacuole and cell cytoplasm. The aim of this research work was to examine the level of *HvTIP1;1* gene expression in barley (*Hordeum vulgare* L.) variety 'Sebastian' under oxidative stress induced by hydrogen peroxide (H_2O_2) and methyl viologen (MV), as well as oxidative and osmotic stress induced by sodium chloride (NaCl).

For this purpose, a reverse transcription reaction was carried out using isolated RNA from barley seedling leaves treated with appropriate doses of compounds inducing oxidative stress. The RT-qPCR technique was used to determine the expression level of the *HvTIP1;1* gene. The effect on *HvTIP1;1* gene expression was studied with using three oxidative stress inducers: hydrogen peroxide, sodium chloride and methyl viologen. The effect of each compound was tested using two doses. Higher doses which was applied resulted in a reduction of expression of the tested gene after 7 hours of treatment, which either remained at the same level after prolongation of treatment to 96 hours as in the case of NaCl, or caused further reduction of gene expression, as well as in the case of $H_2O_2$and MV.

It has been demonstrated that all tested oxidative stress inducers led to reduce the expression level of the *HvTIP1;1* gene; in barley seedlings during the analyzed time points of treatment, which may be related to the function performed by this aquaporin in maintaining homeostasis during abiotic stress. In response to relatively short-acting oxidative stress, plants reduce accumulation/activity of aquaporin HvTIP1;1, so that water transport is inhibited.

Potential of autophagy in equid herpesvirus type 1 (EHV-1) therapy

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Autophagy is one of the most conservative processes in the cell. Viruses can switch on or off this proces, according to their own needs. In this study we investigated how the EHV-1 replication changes in cells treated with different autophagy modulators.

Primary cell cultures of murine neurons were treated with autophagy inductor (rapamycin, 0.1 μM) or inhibitor (chloroquine, 25 μM) for 24 hours. Next, neurons were infected with EHV-1 26 for 2h, 24h or 48h. The level of replication was analyzed using real-time PCR. Colocalization of viral antigens and autophagy markers (p62 and LC3B) was evaluated using confocal microscopy. Cells infected with EHV-1, but without chemical treatment were used as a positive control.

Based on the obtained result from real-time PCR, in cells treated with autophagy modulators, 2 hp.i. (hours post infection) the level of viral DNA was higher, but 24 and 48 hp.i. this level was similar to the level in a positive control. In the cells treated with chloroquine, 48 hp.i. the level of viral DNA increased compared to 24 hp.i., but after incubation with rapamycin level 48 hp.i. and 24 hp.i. was the same. Confocal microscopy analysis showed large amount of viral antigen in cells 2 hp.i. and visibly less antigen 24 and 48 hp.i. Additionally, signals from autophagy markers – LC3B and p62 protein were very strong 48 hp.i. and were observed not only in the cell bodies as 2 and 24 hp.i., but also in neuronal projections.

In conclusion, modification of autophagy process changes the kinetcs of EHV-1 replication. Rapamycin can inhibit viral replication in the late stages of infection. Therefore, it is tempting to speculate, that in the future, modification of autophagy may become useful in antiherpesviral therapy.

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Bacteriophages as a tool for rapid and sensitive bacteria detection

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Bacterial infections are one of the most severe problems in number of fields, such as hospital care (90 000 deaths during hospitalization every year) or food industry (76 million foodborne illnesses annually). Thus, there is a constant need to develop new, fast and sensitive methods for bacteria detection. Recently bacteriophages - viruses that infect bacteria - are gaining growing interest as effective and robust bacteria-sensing elements.

Main goal of my research was to create fast, sensitive and reliable sensor for bacteria detection based on bacteriophages. Bacteria-binding receptors are present only on one end of virion, thus only specific orientation of phages enables effective bacteria detection. For this purpose I used the electric field to orient phages, as they are electrical dipoles. Ordered layers of bacteriophages on gold surfaces were able to detect bacteria in a fast and efficient way.

Application of the constant electric field enabled to orient phages partially and resulted in 4 times higher sensitivity over randomly immobilized phages.[1,2] In further research I utilized the alternating electric field. This approach combined with chemical modification of the gold surface resulted in 64-fold increase in sensitivity and a limit of detection as low as 100 bacteria/ml, *i.e.* in the range of the best sensors based on layers of phages described to date.[3] I not only showed control over density of phages on the surface but also proved their high selectivity in case of real samples. Obtained sensing elements can be applied for selective, sensitive, and fast (15 min) bacteria detection.

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All you need is light – significance of endogenous porphyrins presence in resensitization of *Acinetobacter baumannii* to antimicrobials

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Bacterial cells possess the various porphyrin composition which is strictly connected with heme biosynthesis, electron transport and detoxifying functions. It is well known that this compounds are sensitive to visible light, thus this fact is used in process called Antimicrobial Blue Light Inactivation (aBL). Absorption of photon by light sensitive compound (e.g. porphyrin) leads to excitation and production of singlet oxygen. This radicals leads to cell wall damage, peroxidation of the lipids and proteins which overall results in cell death. aBL is widely applied in eradication of *P. acnes* as well as in the treatment of various skin disorders (e. g. sarcoidosis). It is worth also to mention that still growing antibiotic resistance forces into finding alternative treatment options and such alternative option could be light inactivation which engages porphyrins present in microorganisms.

The main aim of the project was to analyse bactericidal efficacy of aBL towards two clinical isolates of the multidrug resistant human pathogen – *Acinetobacter baumannii*. During experimental procedures, the presence of the endogenous porphyrins in *A. baumannii* clinical isolates (no. 127, 128) was performed and the sub-lethal dose of blue light (λ 415 nm) was determined. Sub-lethal dose was defined as a dose of blue light which leads to the excitation of the endogenous porphyrins and reduced bacterial viability by 0.5-2 $\log_{10}$ CFU/ml, thus it does not lead to complete eradication of the bacterial community. Furthermore, the bacterial cell cultures were irradiated with sub-lethal doses of light and its influence on the antibiotic susceptibility was performed with recommended methodology for synergy testing (e.g. diffusion assays, post antibiotic effect, checkerboard assay).

Overall our experiments confirmed that the presence of endogenous porphyrins in *A. baumannii* and aBL application led to resensitization of tested isolates to antimicrobial agents.

The interaction between T4 bacteriophages and different polypropylene surfaces

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The quality and type of plastic labware is usually ignored as a factor influencing scientific results. However, sometimes it may happen that unexpected errors occur in biological measurements (e.g. in enzymes activity or quantity of DNA). In our research we show that, surprisingly, these errors may be caused by the brand of used plastic labware.

Here we show studies on T4 bacteriophage, which host is Escherichia coli. Two types of plastic tubes were tested: eppendorf tubes (1.5 ml) and falcon tubes (50 ml), all made of polypropylene (PP). We determined which of them are "safe" (i.e. not causing significant decrease in phages activity) and which are not. As a control, we used glass tubes, which did not bring any effect on virus activity. In the case of "unsafe" eppendorf tubes, the decrease of virulence was observed in elevated temperature (50C) (2 orders of magnitude down after 3 days and complete deactivation after 7 days of incubation). In "unsafe" Falcon tubes, the strongest decrease of the number of active phages was seen when the tubes were stirred vigorously (640 rpm) (5 orders of magnitude down after 5h of stirring) and the effect decreased with decreasing stirring speed. Both without stirring and at room temperature (25C), phages activity remained stable for long time (up to 7 days). What is more, it was observed that small amounts of surfactant stabilizes phages against stirring and/or heating.

We performed SEM and AFM measurements to observe plastic surfaces after the experimental processes. These measurements confirmed that after fast stirring active phages are present on the tube wall, even when they are no active phages in the suspension. We propose a hypothesis, that T4 phages are physically adsorbing on plastic walls. T4 phage might be attracted electrostatically to the polymer by its positively charged tail fibers. During stirring or heating the tube, phages are transported faster towards the tube wall and the process of attraction is enhanced.

Effect of sows' feed supplementation with novel synbiotics on faecal concentrations of lactic acid and short-chain fatty acids

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The aim of the research Assessment of three newly developed synbiotics impact on lactic acid, short chain fatty acids (SCFAs) and branched chain fatty acids (BCFAs) production by intestinal microbiota of sows.

Materials and method Danbred sows were reared at a private farm in Poland in high-investment indoor facilities. Animals were divided into 6 groups depending on given feed additive or its absence (synbiotics A, B, C – groups A, B, C respectively; BioPlus 2B® probiotic - group D; Cylactin® LBC probiotic - group E; non - group K, control). The additives administration has begun 10 days before farrowing and sows faeces samples were collected 7 days after piglets birth (17th day of research conducted) and at 24^{th}, 38^{th} and 48^{th} day.

For organic acid concentrations determination, faecal samples were suspended in sterile demineralised water, vortexed, then centrifuged. The supernatant was filtrated through PTFE syringe filters and subjected to high-performance liquid chromatography analysis (HPLC). The concentrations of lactic acid, SCFAs (acetic, propionic, butyric, valeric acids), and BCFAs (isobutyric, isovaleric acids) were detected using the Surveyor HPLC System with the Aminex HPX-87H column.

Results A significant increase in lactic acid and SCFAs (with the exception of propionate), as well as a reduction in BCFAs concentrations in sows' faeces, were observed when synbiotics were used. On the contrary, probiotic preparations did not significantly influence the production of organic acids by faecal microbiota of sows.

Conclusions Innovative synbiotics confer a health benefit on the sows by stimulation of lactic acid and SCFAs production, which can lower pH in intestines and therefore prevent pathogens colonization. Moreover, synbiotics contributed to decreased synthesis of toxic BCFAs.

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Sex chromosomes in lacertid lizards: high stability in spite of reported variability

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Amniotes possess variability in sex determination from environmental sex determination (ESD), where both sexes do not differ in genotype, to genotypic sex determination (GSD) with differentiated sex chromosomes. Some evolutionary models postulate stability of differentiated sex chromosomes and very rare transitions from ancestral GSD to ESD. Lacertid lizards possess highly differentiated ZZ/ZW sex chromosomes. However, sex chromosome turnovers and two independent transitions to ESD were previously reported. In the current study, we examined the homology of lacertid sex chromosomes. We tested linkage to sex chromosomes in several genes previously found to be Z-specific in some lacertids in 45 species from 26 genera including lineages supposed to possess a derived sex determining systems. We found that all tested lacertids share homologous differentiated ZZ/ZW sex determination system, which was present already in their common ancestor living around 85 million years ago. These sex chromosomes are not present in amphisbaenas and teiid lizards, the close relatives of lacertids. Our study demonstrates that inaccuracies in data can influence the outcome of phylogenetic reconstructions of the evolution of sex determination, in this case they led to overestimation of the shifts from GSD to ESD and of the rate of sex chromosome turnovers.

Interaction of serum-derived and internalized C3 with DNA in human B cells – a potential involvement in regulation of gene transcription

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The complement system has originally been viewed as a serum effective system of innate immunity and its components synthetized mainly in the liver. The first evidence for the presence of C3 in human B cells indicating the presence of intracellular C3 in a wide variety of immune cells, among them B lymphocytes. However according to the recent view, the presence of intracellularly detected C3 does not mean automatically that the cells produce the protein themselves. It was concluded that the major origin of intracellularly detected C3 in B cells is the serum since the cells continuously internalize the protein from the extracellular space.

However, in the above publication, the authors did not consider that the B cell receptor itself can activate complement, resulting in covalent attachment of C3 fragments to the cell surface. The membrane-bound and cytoplasmic C3 however can not be distinguished via conventional Western blot methods performed on cell lysates and hence, spontaneous complement activation in the culture media may cause misleading results regarding C3 localization.

Due to the controversial data both on the expression and origin of intracellular C3 in human B cells, in this study we re-investigated the source and localization of C3 in B lymphocytes. Our data show that endogeneous expression of C3 is very low in human B cells and the main origin of intracellular C3 is indeed the serum. Further we prove that serum-derived and purified C3 are able to enter the nucleus of viable B cells and bind to the DNA which suggest its potential involvement in regulation of gene transcription. We suggest that C3/C3a can regulate DNA transcriptions via chromatin remodelling, because of the strong interaction of C3 with histone proteins.

Our data reveal a novel, so far undescribed role of C3 in immune cell homeostasis, which extend the knowledge how complement links innate and adaptive immunity and regulates basic processes of the cells.

Dysregulated miRNA in human plasma as potential biomarkers in Alzheimer's disease

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One of the most common cause of age-related dementia is Alzheimer's disease (AD). Number of people suffering from this progressive neurodegenerative disorder is increasing. It has been reported that in 2015 almost 50 million people were affected by AD-related dementia worldwide and this number is thought to increase to 131,5 million by 2050. That is why Alzheimer's disease is considered to be one of the biggest health concerns in modern society.

Current clinical diagnosis of AD is based on dementia assessment by neuropsychological tests and can be supported by brain imaging biomarkers (amyloid-PET, MRI, SPECT) and by biomarker assays in cerebrospinal fluid that detects levels of amyloid-β and tau and phospho-tau protein. However, these methods require professional and expensive equipment or are invasive and not adjusted for screening of many patients. For these reasons diagnostic method based on biomarkers in blood is highly needful. One of the most promising areas of research for understanding the causes and mechanisms of AD and for development of easily accessible biomarkers is microRNA (miRNA) profiling.

My research concerns development of new diagnostic method for AD based on detection dysregulated microRNAs in human plasma, including possibility to differentiate AD from other dementias. Using qRT-PCR I studied levels of 11 microRNAs which were previously identified in the Laboratory of Preclinical Studies as differential in AD patients in human blood plasma. I compared the levels of these miRNAs in the following patients: 8 patients with AD, 7 suffering from tauopathies and 12 healthy individuals as control group. I have confirmed significant differences in the level of 7 microRNAs in plasma from AD and tauopathy patients versus controls. These results confirmed the potential of the selected miRNAs as AD biomarkers.

What is the connection of tRNA synthesis with storage carbohydrates in *Saccharomyces cerevisiae*?

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Intuitively we may think that differences in the tRNA levels are followed exclusively by changes in the translation rate. However, we observe that activity of RNA Polymerase III (RNAP III) involved in tRNA synthesis, can affect cellularly separated processes such as expression of glucose transporters genes [1], or glycolysis [2].

Yeast mutant strains *maf1-Δ* and *rpc128-1007*, the former with high [3] and the latter with low RNAP III activity [4] show reverse trend in glycolytic activities[2]. Consistent with glycolytic flux, yeast strains with changed RNAP III activity haveeither enhanced ability to store carbohydrates such as glycogen and trehalose during logarithmic growth phase in the MAF1 deletion cells, or demonstrate decreased metabolites levels in rpc128-1007, with respect to wild-type.

This study is the first attempt to elucidate the regulatory mechanism of trehalose and glycogen synthesis in yeast strains accordingly to their RNAP III activity. Surprisingly, we observed increased transcripts levels of several genes involved in storage carbohydrate pathways in *rpc128-1007* despite the diminished content of the end metabolites. To investigate the fate of mRNA in the mutants, immunofluorescence assay using protein markers for stress granules and P-bodies formation was performed. We confirmed that *rpc128-1007* mutant forms cytoplasmic granules, possibly accumulating translationally silenced mRNA, in complex with ATP-dependent RNA helicase Dbp2, but not with poly(A) binding Pab1 protein. On the basis of enzymatic activities of Ugp1 (UDP-glucose pyrophosphorylase) and Nth1 (neutral trehalase), we suggest that the regulation of trehalose biosynthesis in *maf1-Δ* and *rpc128-1007* is controlled on the post-translational level.

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Search for human proteins showing potential interactions with Toxocara canis CTL-1 antigen using yeast two hybrid system

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Toxocariasis is the most commonly occurring zoonotic parasitic disease in the developed countries which is caused by *Toxocara* sp. roundworm larvae. Upon infection in the host *Toxocara canis* larvae release high amounts of molecules which are called TES (Toxocara Excretory-Secretory products). Some of these molecules have already been characterized and are predicted to be involved in immune evasion. One of these is the C-type lectin-1 (CTL-1) antigen, however its function and effect on the host organism have not been clarified.

The aim of the study was to identify human proteins interacting with *T. canis* CTL-1 antigen using the yeast two hybrid system (Y2H). Y2H is an eukaryotic system to detect protein–protein interactions in vivo. The cDNA encoding the CTL-1 antigen was expressed as a fusion to the Gal4 DNA-binding domain (DNA-BD), while a human cDNA library of prey proteins was expressed as fusions to the Gal4 activation domain. Only when two proteins interacted the DNA-BD and AD was brought into proximity to activate transcription of four independent reporter genes. Prey constructs were then isolated from yeast expressing reporter genes. Constructs were then sequenced and nucleotide sequences were analysed using bioinformatics tools.

We selected fifteen proteins showing potential interaction with CTL-1. Among these we found proteins involved in such processes as: regulation of immune responses, cell signalling (expression and regulation of transcription factors, membrane receptors), regulation of cell cycle and apoptosis, protein modification or protein transport.

Discovering protein targets for *Toxocara canis* CTL-1 antigen enables future research aiming to reveal *Toxocara* interaction with the host organism on many different levels of the infection process.

Revealing the unknown. Discovering the biological function of selected plant UDP-glucosyltransferases.

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Coumarins are plant secondary metabolites known for their many advantageous features. They have found a wide range of applications in human life – in pharmaceutical, cosmetic and food industries. In plants, they are involved in defence against various environmental stresses. Scopoletin is a major coumarin in a model plant - Arabidopsis thaliana.

Quantitative Trait Loci (QTL) approach led us to the identification of *A. thaliana* genomic regions which are potentially involved in scopoletin and its glycoside scopolin accumulation within plant roots (1). One of the strongest candidate gene that we have identified in this region was encoding an enzyme the UDP-glucosyltransferase (UGT) with unknown biological function. Since this enzyme have not been described yet, we conducted in vivo and in vitro analysis and tentatively named it scopoletin UDP-glucosyltransferase.

In my studies, I performed comparative analysis of two isoenzymes derived from the parental lines used in QTL mapping - two *A. thaliana* accessions named Columbia-0 (Col-0) and Estland-1 (Est-1). To characterize UGT in planta, the studies using transient expression system in Nicotiana benthamiana were performed. In parallel knock-out mutant lines in Col-0 genetic background were also obtained and characterized. Plants were cultivated in different culture types (*in vitro* on plates and in liquid cultures, in soil) and under various conditions to compare their responses to chosen environmental stress factors.

The phenotypic observations of Col-0 (wild type plants) and ugt mutant lines brought us interesting results, in particular for plants cultivated under osmotic stress. The results of metabolic profiling indicated the presence of interesting differences in scopoletin and scopolin accumulation between Col-0, Est-1 accessions and ugt mutant line. In detail functional characterization of UGT is in progress.

(1) Siwinska et al. (2014) BMC plant biology
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How not to purify exosomes?

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Exosomes are endosomal origin nano sized vesicles, transporting proteins and nucleic acids between cells. They seem to be responsible for cell to cell communication, and even more interestingly also to cancer cells. On their membrane many proteins are localized which allow to penetrate the cellular membrane. They also do not elicit immunogenic reactions. From those reasons they could be an ideal carrier for therapeutic agents. Application of exosomes in medicine is promising but for that their purity has to be excellent. One step purification is not sufficient for this purpose.

HEK293T cell line was cultured and medium was collected. Exosomes were obtained in different purification methods: series of ultracentrifugation, density gradient, precipitation, size exclusion chromatography, proteolytic digestion and concentrators. Purity was determined by Flow Cytometry, Western Blotting, RT-PCR and electron microscopy.

The yield and purity levels differ between the used methods. The precipitation method providethe highest numbers of vesicles, but only part of them were proved to be exosomes. This method also seems to increase aggregation of exosomes, but has no influence of miRNA content and could be availed in diagnostics. The best purity was obtained by combination of different methods inluding series of ultracentrifugation but with very low yield. Density gradient seems to be very promising, but influence of agent used to provide the gradient on many factors is problematic.

The purification of exosomes forces you to go to compromise - either great yield and low purity or struggle with loss of a great percentage of exosomes on each additional step of purification.

Is there any one-size-fits-all method of purifying exosomes? What exosomes on different state of purity can be used for? I will answer this and many other nano importance question.

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Posters

Asymmetric flow field-flow fractionation for the characterization of biologically active molecules and assemblies

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In order for analytical techniques to deliver detailed information on samples that are rather complex (e.g. wide distribution in molecular weight, size, shape, charge and chemical composition), a separation step is often necessary. To this end, a symmetric flow field-flow fractionation (AF4) is a rapidly developing method, due to several advantages that this separation technique has, if compared to more common ones, such as size exclusion chromatography. A wide range of analytes suspended in both aqueous and organic solvents can be processed while also keeping minimizing sample loss and degradation. In its applications AF4 is usually coupled to light scattering and microscopy analytical techniques as well as computer simulation to provide detailed access to the sample properties. To date, AF4 has already been successfully applied in studies ranging from simple macromolecules to more complex assemblies up to entire bacterial cells.

In the present study, AF4 is applied to the understanding of the composition and stability of polypeptides/polymersome hybrid structures, with the final goal of formulating an efficient drug delivery system. Aim of the study is the characterization of the stability and the release properties of such architectures. In particular, pH-sensitive polymersome structures are functionalized with collagenase and analyzed by AF4 coupled to light scattering techniques. The influence of different pH conditions and purification methods (e.g. dialysis and hollow fiber filtration) on the binding of the polypeptide is investigated. The main binding location as well as several structural features of the hybrid structures are studied in deep, as they constitute key parameters when trying to establish an efficient drug delivery system.

The effect of graphene oxide nanofilm on mitochondrial activity and biogenesis in chicken embryo muscle progenitor cells

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The process of mitochondrial fusion and fission is an important mechanism in the process of muscle cell differentiation, as well during disease processes. With maturation, muscle cells have an increasing demand for energy, the mitochondrial network is reprogramming and the energy metabolism of cells is changing - in the production of ATP over glycolysis, oxidative phosphorylation dominates, due to the higher efficiency. During early myogenesis, mitochondria fragmentation is enhanced - DRP1 protein is a main mediator of this process, and subsequent removal of mitochondria via mitophagy. Mitochondrial fusion protein OPA1 is then upregulated, resulting in the reorganization of mitochondrial networks. Finally, the mature muscle cells are replete with new mitochondria. It has been shown that graphene oxide is an appropriate biocompatible material for in vitro studies. Also, graphene oxide stimulates muscle cell differentiation in vitro, however, there are no reports about effect of GO on mitochondria and their dynamics.

The aim of this study was to determine the effect of graphene oxide nanofilm on mitochondrial activity and mitochondrial biogenesis in chicken embryo muscle progenitor cell in vitro.

Analyzes were made regarding cell morphology, viability - with XXT assay, and proliferation - with BrdU assay. Visualization of mitochondria in cell culture was permormed by using mitotracker live stain. The mitochondrial membrane potential was assessed using the JC-10 fluorescent assay, which gives red fluorescence in normal cells, and green fluorescence in apoptotic and necrotic cells. The protein responsible for the fission of the mitochondria – DRP1, was visualized by immunofluorescence. The protein responsible for mitochondrial fusion - OPA1, was identified by enzyme-linked immunosorbent assay (ELISA).

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Campylobacter jejuni C8J0565 plays a key role in the defense against cooper and oxidative stress

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Many bacterial extracytoplasmic proteins are stabilized by intramolecular disulfide bridges that are formed post-translationally between their cysteine residues. This process is facilitated by the Dsb (disulfide bond) family of the redox proteins. These proteins of the model microorganism *Escherichia coli* function in two pathways in the periplasmic space: an oxidation (EcDsbA and EcDsbB) and an isomerization (EcDsbC and EcDsbD) pathway. EcDsbD, integral membrane protein, catalyzes the transfer of electrons across the inner membrane and delivers the reducing power to several periplasmic Dsb proteins with reducing activity. Activity of isomerisation pathway proteins is important in oxidative stress defense in periplasm. The functioning of the Dsb system of *Campylobacter jejuni* is not fully understood.

The aim of this study was to gain insight into biological function of C8J0565 protein identified by *in silico* analysis as potential DsbD protein. To this end the *C. jejuni* 81116 strain lacking *c8j0565* was created by allelic exchange technology. The expected disruption of the chromosomal *c8j0565* locus as a result of the double cross-over recombination event was verified by PCR using appropriate pairs of primers. We evaluated the influence of the lack of C8J0565 on the cooper sensitivity (spot plating assay) and cells defense against oxidative stress induced by H_2O_2. Cells mutated in *c8j0565* were extremely sensitive to cooper and more sensitive to oxidative stress than wt cells.

Our data indicate that CjDsbD plays a key role in the defense against cooper and oxidative stress.

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Evaluation of the membrane support influence on adherent cell function in biohybrid system

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Membrane scaffolds are the structures mimicing extracellular matrix of native tissues supporting the cell grows. Biomaterials, applied for membrane preparaction must meet specific requirements, involving structural, physical, chemical, and biological properties. Recently, scaffolds consisting of natural polymers have been more and more popular. Polysaccharide biopolymers like chitosan and alginate, because of their biocompatibility, biodegradability and bioactivity have a high potential for biomedical applications like drug delivery, or tissue engineering.

The aim of the study was to produce chitosan-alginate scaffolds and evaluate the functionality of two different adherent cell lines – human dermal fibroblasts (HFD) and human osteoblasts (hFOB) immobilized within the membrane. The membranes were prepared by the layer-by-layer technique.Four different nanomembrane systems were studied. They were differing In the number of layer pairs (1 or 3), polyelectrolyte concentration and external layer material. Cells function was assessed on 3th, 7th and 14th day of culture using flow cytometry, fluorescence microscopy and scanning electron microscopy.

Behavior of melanoma cells with different metastatic potential on surfaces modified with proteins from extracellular matrix

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Biomechanical properties of cells, mainly elasticity and adhesion, are very important factors in the search for specific disease biomarkers. Changes in cells properties are closely related to the reorganizationof the cytoskeleton structure. The concentration and structure of individual components of the cytoskeleton determine the mechanical response of the cells to the environment, as well as participate in the interactions with neighboring cells and the extracellular matrix. However, cytoskeletal reorganization (mainly actin filaments and intermediate filaments) in cells can also be triggered by neoplastic transformation. In some types of tumors on the basis of mechanical properties, cancer cells with different metastatic potentials can be even distinguished. This is related to the structural reorganization of the cells during the epithelial-mesenchymal transition. The best example are melanoma cells, which after migration can again increase their stiffness.

The aim of the study was to examine the biomechanical properties of melanoma cells from both primary (radial growth WM35, vertical growth WM115) and metastatic (skin WM266-4, lungs A375-P) phases grown on modified surfaces. The surfaces were modified with proteins occurring in extracellular matrix (ECM) like fibronectin, collagen or laminin. The methods used for the study were: atomic force microscopy to study cells' elasticity; fluorescent staining to show differences in cells actin cytoskeleton and the degree of cells adhesion to the modified surfaces,crystal violet staining to observe cells morphology and to apply the cell adhesion assay.The preparedglass surfacemodifications with ECM proteins had a visible effect on the cellsmechanical properties and adhesion, due to forcing the cells to use specific membrane receptors to be able to adhere to different substrates.

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C3A cell line with restored urea cycle as a promising source of cells for BAL devices

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Liver diseases that lead to its failure are one of the most frequent causes of death worldwide. Taking into account liver's complexity, there are no effective medical solutions for acute or acute on chronic liver failure. The only long-term cure for acute forms of liver failure is orthotropic transplantation. Unfortunately donor shortage is a main problem of this therapy. According to the Organ Procurement and Transplantation Network approximately 14 000 patients are currently on the transplant waiting list in the USA and circa 1 200 patients die each year while waiting. One of the most promising alternative for liver transplantation are bioartificial liver devices. They constitute a bridging therapy for patients waiting for the transplantation. ELAD, the most advanced device among BALs, came to the third phase of clinical trials, but did not live up to expectations.

The ELAD utilizes the C3A cell line with non-functional urea cycle and this can be a reason for the clinical therapy failure. Therefore, we proposed a genetic modification using lentiviral vectors and obtained C3A cells with the restored urea cycle (C3A AO). These cells can be subsequently use in BAL devices.

C3A AO in standard conditions are characterized by increased albumin and urea production compared to their unmodified counterparts. To mimic *in vivo* conditions, C3A and C3A AO cells were cultured in capillary flow bioreactors. During the experiment the most crucial parameters such as cell viability, glucose consumption as well as albumin and urea production were measured and then compared.

Although cells did not attach to the capillary membranes, we observed increasing albumin production during the experiment. We were not able to measure other parameters because of the death of a significant number of cells. As a flow bioreactors have a high application potential, research towards improving cells' adhesion to the capillary surfaces and as a result to conduct long-term culture will be continued.

Quantitative analysis of Fe distribution in mouse tissues after supplementation with ZnO: Fe nanoparticles, comparison of Tissue-Facs and Micro Image methods

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With current iron deficiencies, new routes are sought for its supply, which would increase its absorption from the digestive system and reduce potential toxicity. One of the exits in this case may be nanoparticles, which are characterized by completely different properties from current drugs, there are other ways of distribution of these particles, they easily overcome barriers in the body. You can also modify the size, composition and their biodegradability. During the research described in this thesis, new-generation nanoparticles - ZnO were used, which are biodegradable. The experiment was carried out in vivo on a mouse model. The Pearl's staining was used to visualise the iron aggregates in tissue. It was examined whether the application of ZnO: Fe nanoparticles influences the increase of its concentration in the tissues of the brain, spleen and liver, in relation to the control. Aim was to evaluate the influence of iron with the nanoparticles on iron levels comparing two quantitative methods Tissue-Facs and Micro Image. Current methods of quantitative research based on chromatic dyes are very absorbent and require a lot of time, which is why methods are needed that would allow for greater automation of this process. One of the solutions may be Tissue-Facs cytometer. Results showed the accumulation of iron in mice after 24 hours after oral administration of ZnO: Fe nanostructures was detected using the Micro Image software in key organs, which are the main storage and transformation sites of this element: in the liver and spleen. There was a tendency to accumulate iron around the blood vessels in the spleen, which may indicate the transport of NP: Fe from the general blood stream to the tissue observed using the Micro Image method. The Micro Image software has proved to be a better tool for quantitative iron analysis in chromatic dyed tissues, compared to the Tissue-Facs scanning cytometer.

Efficient vesicular trafficking protects yeast *Saccharomyces cerevisiae* genome from fragmentation

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With time, more and more cellular mechanisms are considered as significant for the genome maintenance. Results of our previous genome-wide studies had drawn our attention to vesicular trafficking as a process that contributes to genome preservation. Among 63 strains lacking various vesicular trafficking genes that were engaged in the protection of cells against stress of DNA double strand breaks or involved in spontaneous mutagenesis, we found several strains that showed highly increased DNA fragmentation visible in a single cell gel electrophoresis (comet assay). These strains exhibited also other phenotypes linked to genome instability. Firstly, they showed increased sensitivity to exogenous genotoxic stress (e.g. upon zeocin or MMS treatment). Secondly, in these strains, the recruitment of Rad52-YFP to DNA repair foci was decreased, which suggests impaired DNA double strand breaks recognition or defects in DNA repair. Thirdly, these strains displayed increased frequency of Rfa1-YFP spontaneous foci formation, as compared to control strain. Moreover, in contrast to WT control, the rate of Rfa1-YFP foci formation was not elevated significantly in response to genotoxic stress. Finally, the flow cytometry analysis of mutant cells stained with propidium iodide indicated DNA content aberrations. Collected data imply moon lighting role of vesicular trafficking proteins in cellular response to DNA damage.

Western diet accelerates neuroinflammation in mice model of Alzheimer's disease

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Western diet (WD) is a dietary pattern enriched in high saturated fatty acids like cholesterol and containing higher levels of simple carbohydrates. WD is currently known as a risk factor of systemic inflammation, metabolic syndrome and obesity. Moreover, several studies suggest that WD driven changes could lead to Alzheimer's disease (AD), but the underlying mechanisms remain unclear. We aim to verify hypothesis that long term WD feeding impairs brain homeostasis and leads to neuroinflammation at early pre-symptomatic stages of AD development.

To this aim, we analyzed brain tissue from mice model of familial form of AD, the transgenic mice (Tg2576) expressing human Swedish mutation of amyloid precursor protein (APP_{swe}). To induce metabolic syndrome and systemic inflammation, APP_{swe} mice were fed with WD, or/and intraperitoneally injected with Lipopolysaccharide (LPS) as a control factor inducing systemic inflammation. To determine the impact of WD and LPS on the sequence of events in brain, the analysis was carried out in three age groups (4-,8-,12-month old mice).

We found that WD feeding induces astrogliosis and proinflammatory cytokines dysregulation in the brains of young APP_{swe} mice. Therefore we postulate that WD may be consider as an important factor resulting in neuroinflammation at the early stage of AD development. This hypothesis is supported by pro- and anti-inflammatory cytokines profiles in APPswe mice brain.

Obtained results indicate that consuming of WD accelerates and intensifies the astrogliosis observed during AD development and suggest that WD can be considered as a common civilization risk factor of AD progression.

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Rapid identification of biofilm-forming *Staphylococcus pseudintermedius* strains isolated from dogs

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Staphylococcus pseudintermedius is a skin and mucous membranes commensal in dogs. However, it can become an opportunistic pathogen, and is frequently isolated from canine pyoderma. Moreover, staphylococcal species are the most commonly isolated from canine ear and wound infections and canine urinary tract infection. *S. pseudintermedius* produces a variety of virulence factors, including coagulase, protein A, proteases, enterotoxins, cytotoxins and exfoliative toxin. Additionally, *S. pseudintermedius*has also been shown to form biofilms. Biofilm protects bacteria against host immune responses and antimicrobial agents. Infections caused by biofilm-forming *S. pseudintermedius* can be difficult to control due to these protective mechanisms. There is a need to use a rapid identification method to determine biofilm formation in *S. pseudintermedius*in order to adjust the treatment.

The aim of the study was to determine the presence of the intercellular adhesion *icaA* and *icaD* genes in biofilm-forming *S. pseudintermedius* strains.

The research material consisted of 11 *S. pseudintermedius* strains isolated from 8 out of 51 dogs. Swabs were taken from outer ears and skin.All *S. pseudintermedius* strains were classified as biofilm-forming by phenotypic observation in polystyrene microtiter plates. The presence of *icaA* and *icaD* genes were determined by PCR.

The presence of *icaA* and *icaD* genes was found in 8 isolates out of 11 (72%), although both variants were detected only in 55% (6/11) of strains. On the other hand, 9% (1/11) lackedboth target genes.

The occurrence of the *icaA* and *icaD* genes may be associated with the phenotypic ability of *S. pseudintermedius* to form biofilms.Nevertheless, the lack of *ica*A and *ica*D genes sometimes occurs and does not affect the formation of biofilm. Research will be continued on larger group of *S. pseudintermedius* strains to justify the use of PCR as a rapid identification method of biofilm formation.

Analysis of anticancer drug cytotoxicity on the spatial breast cancer model in the Lab-on-a-chip system

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Laboratory tests of drugs with a potential anticancer effect are mainly performed on two-dimensional (2D) cellular models. Such two-dimensional models are not similar to structures that build tumor tissue *in vivo*. The development of tissue models (3D) that mimic the conditions of the living organism is currently one of the main goal of cellular engineering. The *Lab-on-a-chip* systems may be useful in the creation of 3D cell models. They provide dynamic fluidic conditions, which are a natural factor, stimulating cells for growth and proliferation.

In our work, we designed a new microfluidic system for multilayered 3D cell culture. The microsystem consisted of two layers of poly(dimethylsiloxane) and a porous polymeric membrane made of poly(ethylene terephthalate) (PET). We used two cell lines in the experiments: human breast fibroblasts (HMF) and breast cancer cells (MCF-7). The microsystem geometry enabled cell culture on both sides of the porous membrane, ensuring spatial arrangement of the cells and constant contact of the extracellular matrix (ECM) of both cell types.

The designed microsystem was used to study the proliferation of non-malignant and cancer cells and to evaluate cell viability after a chemotherapy procedure. The cytotoxic properties of doxorubicin (DOX) against breast cancer in the concentration range of 0-100 μM were examined. According to the obtained results, the cell viability decreased with increasing concentration of drug. Cell viability was 71.6%, 52,4%, 30.7% and 23.9% for 0.1, 1, 10 and 100 μM of DOX, respectively. Viability was assessed by differential staining of the cells using fluorescent dyes: Propidium iodide and Calcein-AM.

Our research confirmed that the properties of the PET membrane allowed the adhesion of breast cells on its surface. In addition, the use of microfluidic conditions promoted the proliferation of the cells cultured on the membrane. The microsystem could be useful as a tool for anticancer drugs testing.

Safe and natural – antimicrobial compounds produced by lactic acid bacteria against pathogenic *Escherichia coli*

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Protection against pathogenic microorganisms is still considered as one of the most important challenges for food production. Among the most severe etiological factors of food poisoning are distinguished enterotoxic and enterohemorrhagic *Escherichia coli* strains. Increasing consumer awareness on artificial compounds and additives in food drives food producers to discover alternative solutions prolonging the shelf life of products and avoiding contamination with pathogenic microorganisms. One of these methods is using natural food preservatives –bacteriocins, proteinaceous or peptidic antimicrobial compounds produced by lactic acid bacteria (LAB). As they are easily digested in human gastrointestinal tract, bacteriocins are generally considered as safe (GRAS) for the use as food additives. Furthermore, they have no carcinogenic effects and are stable in a wide range of pH and temperatures, which is crucial in a food production. The aim of the study was to determine the antagonistic activity of LAB and their potential for the production of bacteriocin that is active against enterotoxic and enterohemorrhagic *E. coli*.

In this research 7 strains of lactic acid bacteria were isolated from plain yoghurts. Antagonistic effect of LAB towards 9 pathogenic *Escherichia coli* strains was determined by spot-test method. For the detection of antimicrobial proteinaceous compounds, the modified well diffusion method was performed using supernatants obtained from pure LAB cultures and proteolytic enzyme (papain). Obtained results showed that lactic acid bacteria cause an antagonistic effect towards enterotoxic and enterohemorrhagic *E. coli* strains. Moreover, some of isolated LAB strains produced an antimicrobial proteinaceous compounds against these pathogens. Isolated LAB and their antimicrobial metabolites may find an application in food production by protecting it against pathogenic strains of *E. coli* and reducing the use of artificial food preservatives.

Pathogenic Legionella protein kinase families

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The intracellular pathogen *Legionella*, resides inside the cell of its eukaryotic host. The deadly *Legionella pneumophila* secretes a set of 300+ effector proteins and via this establishes a comfortable niche for itself. When delivered into the host cell, effector proteins hijack the signalling pathways. Most of the effectors are still poorly understood or completely uncharacterised. Also, several distant homologues of eukaryotic signalling proteins were discovered among them recently.

Here, we focus on the effector proteins of *Legionella* genus, which have fold similar to Protein Kinase-like clan (PKL). We will show bioinformatics analysis of the known PKL effectors, e.g. MavQ, LegK1-4, LepB and novel PKL effectors discovered by us in recent years. Next, we compare them to non-effector *Legionella* kinases and to human host kinases and discuss the evolutionary relationships of the novel enzyme families to the known distant relatives using sequence and structure data

Novel *Legionella* PKL effector families are an example how bioinformatics algorithms for three-dimensional structure prediction and remote homology detection can help in finding novel enzyme families.

Those analyses indicate that horizontal gene transfer plays an important role in the host-pathogen arms race in the cell interactions world.

Application conjugate of enkephalin and temporal DAL-PEG-DK5 in the treatment of periodontitis

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Antimicrobial peptide (AMPs) DAL-PEG-DK5 is composed of lysine-rich derivative of amphibian temporin-1CEb (DK5) and dalargin (DAL), the synthetic analogue of human enkephalin (DAL) that linked by PEG linker. Antimicrobial peptide can kill directly gram negative and gram positive bacteria including bacteria which caused periodontitis such as *Streptococcus gordonii*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* that present in early, middle, and late periodontitis. Periodontitis is an inflammation disease which causes damage the soft tissue and bone supporting the teeth. The periodontitis happened when there is an accumulation of bacteria (known as periodontal bacteria) builds up the plaque and is left undisturbed on the teeth, then its form biofilm.

The aim of this experiment is to evaluate the potential use of DAL-PEG-DK5 antibacterial peptide conjugate in the treatment of periodontitis. Our result has shown, the minimum inhibitory concentration in the case of *P. gingivalis* W83 and *F. nucleatum* 25586 is 25μg/ml, further incubation (3 hours and 6 hours) does not increase the effect of the peptide to kill the bacteria. On the basis of the live-dead test - 1 hour of incubation of bacteria with the peptide at a dose of 25μg /ml causes 40% mortality. The best time to give antimicrobial peptide DAL-PEG-DK5 to inhibit the formation of biofilms is to be given at the same time at the beginning of biofilm formation. At concentration 50μg/ml peptide inhibits a half of biofilm formation of *P. gingivalis*. Moreover, the antimicrobial peptide DAL-PEG-DK5 induces aggregation of *P. gingivalis* endotoxin.

Conjugates of antisense oligonucleotides with boron clusters: new material for Boron Neutron Capture Therapy

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DNA nanotechnology is a branch of technology that exploits nucleic acids' ability to self-assembly in order to construct nanostructures with specific properties. There are numerous potential applications of DNA nanostructures including those in diagnostics and therapy of human disorders [1]. Based on our previous studies on boron clusters as modifying units for nucleic acids [2,3], conjugates of the epidermal growth factor receptor (EGFR)-directed antisense DNA oligonucleotides modified with boron clusters [o-carborane, $C_2B_{10}H_{12}$; dodecacarborane, $B_{12}H_{12}^{2-}$ and metallacarborane, $[Fe(C_2B_9H_{11})_2]^-$] were obtained and tested as potential agents in antisense and BNCT therapy [4,5].

In this communication, we present an application of DNA-functionalized boron clusters (oligopods) as building blocks for nano-construction of therapeutic nucleic acid systems. Thus, tri-substituted o-carborane, bis-functionalized with EGFR-targeted sense or antisense oligonucleotides were obtained by solid phase method. The complementary dipods were self-assembled to nano-structured complexes which were visualized by the non-denaturating polyacrylamide gel electrophoresis (PAGE), atomic force microscopy (AFM) and cryo-transmission electron microscopy (Cryo-TEM). Their silencing activity, stability against exo- and endo-nucleases as well as melting properties were investigated.

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Lab-on-a-Chip system integrated with nanofibrous mats for hypoxia symulation

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Ischemic heart disease (IHD) is the main cause of death around the world. The number of people suffering from cardiovascular diseases increase every year. Myocardial necrosis, caused by hypoxia is one of the symptoms of IHD. In the case of IHD, the most effective method of treatment is heart transplantation. However, this method is limited due to the number of donors. Therefore, it is necessary to improve current therapies. Detailed understanding of the disease at cellular level is an important factor in the development of a new treatment method. The answer to this need can be cellular or tissue models. In addition, the miniaturization allows to create the cellular model in *Lab-on-a-Chip* systems. The microsystems enable cell culture under conditions that better imitate *in vivo* conditions, compared to standard multi-well plates.

We present a microfluidic system integrated with nanofibrous mats. The nanofibrous mats are made of polyurethane (PU) using solution blow spinning method. Nanofibers are intended to be a scaffold for cardiac cells culture (rat cardiomyoblasts, H9C2). The use of nanofibrous mats should also cause parallel cell arrangement, which may imitate the myocardial tissue *in vivo*. The presented microsystem consist of two layers, made of poly(dimethylosiloxane), between which the nanofibrous mats are placed. The network of microchannels and culture microchambers in the top layer allow for cell culture under different conditions at the same time. There are microgrooves for nanofibrous mats in the bottom layer. Moreover, the arrangement of the culture microchambers corresponds to the arrangement of wells on a multi-well plate. Therefore, it is possible to perform quantitative analysis by using multi-well plate reader. The designed microsystem can be used as a tool to study the effect of hypoxia on cardiac cells.

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3-Bromopyruvate – an anti-carcinogenic compound with an activity against *Helicobacter pylori*

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Helicobacter pylori is a bacterium colonizing 60% of people in the world, which is associated with the development of gastritis, gastric ulcers and gastric cancers. One of the main challenges of therapies directed against *H. pylori* is the increasing resistance of this bacterium to the antibiotics used. Therefore, the recommendations of the World Health Organization report the need to search for new substances with activity directed against this bacterium. The substance tested in our research was 3-Bromopyruvate (3-BP), an anti-carcinogenic compound with antimicrobial activity. The research was carried out on clinical and reference *H. pylori* strains, characterized by a diversified profile of resistance to antibiotics. Determination of antimicrobial activity was carried out by a disk-diffusion and microtitration method. The studies were extended to analyze the viability and morphological variability of *H. pylori*. The morphology was assessed by microscopic techniques (light and scanning electron microscopy), whereas viability was determined by a Live/Dead staining combined with the measurement of fluorescence. For 3-BP, the presence of bactericidal activity was observed, which was demonstrated both independently and in combination with selected, clinically used antibiotics. In addition, it was noted that the presence of 3-BP increased the effectiveness of antibiotics, reducing the concentration required to kill H. *pylori*. The decrease in bacterial viability was proportional to the applied concentration of 3-BP with the fastest total reduction in viability observable after a 2-h incubation of *H. pylori* with a four-fold minimal inhibitory concentration (4×MIC). It was noticed that after a one-day incubation of *H. pylori* with MIC of 3-BP most of the cells were degraded, regardless of the morphological form presented. These results indicate the potential for using 3-BP as an antimicrobial agent with an activity against *H. pylori*.

Immune escape and stem cell markers profile of breast cancer cell lines in the context of epithelial-to-mesenchymal transition

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Epithelial-to-mesenchymal transition (EMT) allows metastasis formation by cancer cells. As a result of EMT cells acquire mobile and invasive phenotype. It was also shown on lung cancer that post-EMT (mesenchymal) cells have decreased immunogenicity, what was connected with decreased expression of antigen presenting complexes and immunoproteasome (IP) subunits. IP is responsible for peptides processing to be presented on the cell surface, what in the case of cancer cells results in activation of immune response. Tumour cells can also directly suppress T cells by sending inhibitory signal via upregulation of PD-L1 and PD-L2 on the cell surface. Post-EMT cells can also have self-renewal properties due to stem cells markers expression.

We hypothesized that expression of genes coding for IP subunits: *PSMB8*, *PSMB9*, *PSMB10* and antigen presenting complexes: *TAP1*, *B2M* are lower in mesenchymal than in epithelial cells, whereas the opposite situation should be observed for *PD-L1*, *PD-L2* and stem cell markers (*CD44*, *NANOG*). Four breast cancer cell lines (MCF7, T47D, MDA-MB-231, HCC-1806) of different molecular subtypes were classified to different EMT states based on the expression of *EpCAM, MCAM, CDH1*, *CK19*, *VIM, SERPIN, PLS3* genes. mRNA was isolated from cell cultures and reverse transcribed, next relative expression of the genes was tested with qPCR.

Epithelial MCF7 cell line showed the lowest expression of genes involved in immune escape (*PSMB8, PSMB9, PSMB10, B2M, TAP1*), whereas mesenchymal MDA-MB-231 and triple negative HCC-1806 cell lines showed increased expression of PD ligands. Also, increased *CD44* expression in HCC-1806 cell line was observed. Therefore, our results indicate that in breast cancer post-EMT cells might actively inhibit the immune system due to increased level of PD ligands, whereas epithelial MCF7 cells decrease their immunity. Further studies are needed to investigate the role of immune escape-related genes in the context of EMT.

Application of in silico methods in structure analysis of supramolecular complexes of cyclodextrins with active pharmaceutical ingredients used in the treatment of pulmonary hypertension

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Pulmonary hypertension (PH) is a relatively rare disease with a severe course characterized by the presence of elevated blood pressure in the pulmonary circulation. The most important groups of drugs used in PH therapy include: phosphodiesterase type 5 inhibitors, endothelin receptor antagonists and prostanoids. The problem is necessity to maintain a constant concentration of active pharmaceutical ingredients (API) in the patient organism associated with a frequent dosing of the drug (e.g. three times daily). For this reason, long-acting forms of drugs that would release API in a controlled manner are sought. The ideal solution in the treatment of PH seem to be complexes of API with cyclodextrins (CDs).

Currently, the interest of the pharmaceutical industry in the use of advanced computational techniques in the seeking of new therapeutic solutions is steadily growing. The dynamic development of in silico methods reduces costs and shortens the time from the idea to the invention of a new drug form. The methodology of the rationalized drug design includes aspects related the use of computer techniques for parameterization of supramolecular complexes of API, estimation of their physicochemical properties, and prediction of their stability and biological properties.

The purpose of this work was analysis of the structure of supramolecular complexes of API used in PH with various types of CDs, e.g. α-CD, β-CD, γ-CD and modified CDs by density functional theory (DFT) method and the selection of the most energetically stable complex. Geometry optimization was performed using Gaussian 16 program for vacuum and using polarizable continuum model (PCM) simulating the aqueous environment. Afterwards, the stability of the complexes was compared and the ability to attach an API molecule was evaluated based on the analysis of the results. In the case of many drugs, depending on the size of the CD cavity, the API assumes a different position in the complex.

Escherichia coli biofilms and their enzymatic fingerprinting

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The number and activity of microorganisms are very important factors which decide about thequality of drinking water. The occurence of biofilms in distribution systems of water leads toa significant deterioration of its quality. Bacteria are the first colonizers of the surfaces beingin contact with water. They produce a wide range of enzymes, both intra- and extracellular.Their release into the environment is the base of interactions between microorganisms andresults in undesired effects like biocorrosion or changing of organoleptic characteristics ofwater. The research was conducted to assess the spectrum of biofilm enzymatic activitydepending on environmental conditions. Bacterial strain *Escherichia coli* ATCC 8738 wasused as the biological material. The dynamic of biofilm formation during 3 weeks ofincubation at temp. 25°C in 3 types of culture media (various availability of nutrition) on 2 different abiotic surfaces (glass, PET) was assessed using swabbing and plate count method.The enzymatic activity of mature biofilms was examined using commercial tests API Zym.The biggest biofouling occured in the poor culture media, measured by the number of livingcells (jtk/cm^2) on glass and PET was equal $7{,}68 \times 10^6$ and $4{,}8 \times 10^5$, respectively, andmeasured by a relative adhesion factor (%) was 4,28 and 0,42. Biofilms formed in suchconditions were characterized by a meaningful enzymatic activity, especially: β-glucuronidase, esterase, alkaline phosphatase, naphyhol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase.

Stereoselective reduction of monohydroxy flavanones to corresponding flavan-4-ols by the yeast *Rhodotorula glutinis* KCh735

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Yeasts from genus *Rhodotorula* are common inhabitant of many environmental niches, due to remarkable ability to assimilate nitrogenous compounds can be dissected from soil, water, food and even air. *Rhodotorula* can be easily detected and identified, as these yeast are known of pinkish colour which is associated with biosynthesis of a group of carotenoids such as torulene, torularhodin and β-carotene. The widespread occurrence in the environment and ability to grow on minimal media allows us to conclude that they have a rich enzyme apparatus, especially that some strains are able to metabolise hydrocarbons or even phenol, what indicate a great potential of wide application in biotechnology, biotransformations or bioremediations.

Flavonoids are group of compounds naturally occurring in plants, thus are inherent part of daily human diet. Undeniable health benefits are related to their antioxidant activity. Recently, *Rhodotorula glutins* KCh735 were descried as a very useful catalyst to transform many natural C-7 hydroxy flavonoids to corresponding C-8 hydroxylated derivatives, therefore forming catechol moiety and greatly increasing the antioxidant activity. Hence, microbial transformations of flavonoids allow to obtain interesting - more active compounds.

The purpose of the study was to test the yeast *R. glutins* KCh735 as a catalyst to transform a range of monohydroxy flavanones, and to verify the regioselectivity of C-7 hydroxylation. Interestingly, only C-7 hydroxy flavanone was hydroxylated at C-7 position. In case of all other tested substrates we detected products of asymmetric reduction at C-4 carbonyl group of which two corresponding diastereoisomers were formed. Depending on the position of the hydroxyl group in flavanone derivatives, reaction proceeded with different effectiveness and product ratio. Combination of NMR, HPLC, and chiral HPLC allowed to determine the structure of resulting compounds and the stereoselectivity of the reaction.

Preliminary analysis of transcriptome indicates potential mechanisms involved in carrot response to salt stress

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The plant material were salt sensitive double haploid line DH1 and salt tolerant Asian landrace DLBA. Plants were grown in peat substrate and sand, with EC = 0.2 mS·cm $^{-1}$ for control and EC = 3 mS·cm $^{-1}$ for stress induction. To maintain soil salinity plants exposed to salt stress were irrigated with 100 mM NaCl solution. For each genotype, organ and treatment RNA probes were prepared from 3-8 individual plants, in three replicates, and sequenced with Illumina 4000.

Transcriptome analysis showed that in the tolerant plants (DLBA) 727 and 272 genes were differentially expressed (DEG), in leaves and root respectively. Among them, 448 and 214 were upregulated, 279 and 58 were downregulated, in leaves and root respectively. In the salt sensitive line (DH1) much lower number of DEGs was identified in leaves (90) than in roots (1176). In this line, 48 and 918 genes were upregulated while 42 and 258 were downregulated, in leaves and roots respectively. The analysis of DLBA transcriptome showed, among others, upregulation of a proline-rich, 33 kDa extensin-related protein gene, a 14 kDa proline-rich protein DC2.15-like gene and downregulation of proline dehydrogenase gene in plants exposed to salts stress. Also, the expression analysis showed downregulation of catalase-like protein and catalase isozyme 1-like genes in leaves. These findings were congruent with a higher proline content and lower catalase activity in tolerant DLBA plants. Additionally, the expression of two other genes was identified in tolerant DLBA plants only. Transcripts of MNS3 gene encoding AT5ptase, a protein involved in regulation of reactive oxygen species formation, was observed in leaves and roots of DLBA growing in saline soil. These plants transcribed also the TAF10 gene encoding a transcription factor involved in the adaptation to osmotic stress, but the transcripts were present only in leaves.

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Phylogeny of type I and II J Domain Proteins using Bayesian and Maximum Likelihood methods

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Hsp70 chaperone system participates in many protein folding and refolding processes ranging from nascent folding, do protein disaggregation and remodeling. Much of this multifunctionality is achieved due to interaction with J Domain Proteins (JDPs), a diverse family of co-chaperones. Main function of JDPs is regulation of Hsp70 ATPase activity via its highly conserved J-domain as well as substrate selection and presentation through variable C-terminal domain. Such strong partnership would suggest a co-evolution of these proteins. Previous studies have shown distinct origins of at least four groups of eukaryotic Hsp70s that share a common intracellular localization. These grous include proteins acting in cytoplasm, endoplasmic reticulum, mitochondria and plastids. Here we show that JDPs share similar evolutionary history using Bayesian an Maximum Likelihood methods.

On the trail of new markers of pacemaker cells

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Nearly 1% of newborn children suffer from congenital heart disease (CHD). Conditions associated with the disorder of the heartbeat rate are called arrhythmia, of which four main types exist - tachycardia, bradycardia, atrial/ventricular fibrillation, and chamber bigeminy. Knowledge about the molecular basis of these diseases remains limited and requires research on the factors that regulate heart development and function. The cardiac conduction system (CCS) is responsible for generating and proper spreading of the electrical impulse at the heart. The signal produced by the pacemaker cells located in the sinoatrial node (SAN) is propagated over the entire heart, causing the contraction of the atria first, followed the ventricles. Abnormalities in SAN development may lead to cardiac arrhythmia and sudden death. Although some of the genes responsible for the development of SAN were known, including transcription factors like Shox2, Tbx18, Tbx3 or Isl1, many other underlying genetic causes of various forms of arrhythmia remain unknown. The zebrafish (*Danio rerio*) is a good model for studying heart biology due to its rapid external embryogenesis and conserved cardiac electrophysiology to that of humans. In a previous analyses, potential candidate genes implicated in pacemaker development were identified through RNA-seq carried out on cells isolated from ET33mi59b transgenic line which expresses EGFP in the sinoatrial junction containing pacemaker activity. In order to functionally validate these candidate genes, we first studied their expression pattern in the developing heart via in situ hybridization. We will then perform further functional analysis using Morpholino and generate mutant lines of these genes using the CRISPR-Cas9 system.

Distribution of lacertidae in the danube – dniester region (Ukraine)

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Currently, the problem of biodiversity conservation is particularly acute for the southern part, in Ukraine. The state of environment's protection potential in the Danube-Dniester region is of particular interest in. It is a part of the Azov-Black Sea eco-corridor, which is one of the most important for the conservation of Ukraine's biodiversity (Shcherbak, 1988). Modern data on the herpetofauna of this region is very limited. Representatives of Lacertidae family are the most interesting objects for reptiles' studying within this region. They often become objects of morphological and environmental research, aimed at studying the population structures, bioeographical distribution, both in nature conservation areas and anthropogenically transformed territories [Karmyshev, 2002; Nekrasova, 2013]. The aim of this work is the detection of places of Lacertidae lizards' modern distribution in the Danube – Dniester region. For this purpose, in August 2017 an expedition was conducted. The research was carried out by the route method with harmless animals' photographing methods. As a result, 5 lizards' species were found: *Podarcis muralis* (Laurenti, 1768), *Lacerta viridis* (Laurenti, 1768), *Podarcis tauricus* (Pallas, 1814), *Lacerta agilis* (Linnaeus, 1758), *Eremias arguta* (Pallas, 1773). *P. muralis* was found in only 3 localities near the city of Reni: in the abandoned transportation complex in front of the seaport, the first and second passage through the city channel. *P. tauricus* was found only near the city of Reni, Lake Kahul (Nahirne and Orlivka villages), Kuhurluy and Yalpug lakes. In the Danube-Dniester region, the number of green lizards is quite high. It is distributed throughout the region, but the territorial structure of their populations is mosaic and the number of lizards towards the Dniester River (Odesa region) decreases. *L. agilis* is found only between the Dniester river and the Izmail district near the urbanized territories.

Defining lamin-associated proteome in *Drosophila melanogaster*-a new approach to the molecular basis of laminopathies

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Lamins are a major part of a nuclear filamentous network called lamina. It is known that lamins are responsible for maintaining the normal structure of the nucleus, regulation of transcription and the organization of chromatin. Mutation in genes coding for those proteins may cause diseases called laminopathies. Up to now, over 350 mutations in lamin gene have been identified that results in at least 30 types of diseases hence it is very important to examine the exact mechanism that causes this abnormality.

To examine the exact molecular process occurring during these disorders the cellular stress imitating the disease was performed in *Drosophila melanogaster* model system. The major aim of this work is to identified potential protein components associated with lamin and moreover to investigate changes in lamin itself (such as post-translational modifications eg. phosphorylation). To achieve the goal, techniques such as co-immunoprecipitation and mass spectrometry analysis were used.

Our data suggests that there is a significant difference between lamin-associated proteome in normal and stress conditions. To investigate whether there are differences in lamins itself the solubility in increasing salt concentration was examined. The changes in lamin association with chromatin and solubility were checked in normal conditions and after heat shock induction on embryos and Kc cells. We showed that lamin displays to be better soluble after heat shock induction together with its potential partners.

Laminopathies due to very diverse symptoms are an extremely heterogeneous group of diseases. To get the closer look at understanding the molecular mechanism of this disease it is extremely important to identify lamin-associated proteins which may be involved in described processes. We showed that there is a significant difference in proteome profile between lamin-associated protein in both conditions as well as the solubility of lamin Dm before and after heat shock.

Interactions of recombinant TRF1 and TRF2 shelterine proteins with telomeric DNA

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The human hTRF1 (Telomeric repeat-binding factor 1) and hTRF2 (Telomeric repeat-binding factor 2) belong to the shelterine complex protecting telomeres. The proteins recognize the telomeric sequence of DNA.

This structure ensures the stability of the chromosome by rolling the telomeric DNA into two specific loops and protects against undesirable activities of the DNA repair cycle. The hTRF1 and hTRF2 are the one of the shelterin complex that exhibit high affinity for double-stranded telomeric DNA and bind to DNA as a homodimers by TRFH domain. The hTRF1 is a negative regulator of the length of telomeres. TRF1 along with TRF2 normally prevents telomerase from adding more telomere units to telomeres.Additionally the hTRF2 covers and protects the ends of the chromosomes. The removal of hTRF2 from telomeres results in the loss of the cell's ability to discriminate the natural ends of DNA from cracking and cutting off the single-stranded chain at the end of 3‘ hanging, leading to telomere dysfunction.

Both hTRF1 and hTRF2 contain the TRFH homodimerization domain and the Myb type domain which specifically recognizes and binds telomeric DNA. The hTRF1 N-trminus is rich at the acidic residues however the TRF2 is rich at the alkaline residues.

We are overexpressed both the recombinant TRF1/2 in *E.coli* system. Secondly we confirm the ability of specific binding to telomeric DNA by recombinant TRF1 and TRF2 proteins. The aimed of our study is to demonstrate the importance of the use of recombinant TRF1 and TRF2 proteins to study in vitro protein-DNA interaction model. It will allow testing of chemical compounds which may be a potential solution in the fight against cancer cells.

Mechanical properties of erythrocytes treated with multi-walled carbon nanotubes functionalized with carboxyl groups

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Carbon nanotubes due to their unique properties are interesting materials for electronics, nanotechnology, material science etc. In medicine they could be applied as drug containers and carriers. The aim of the study was to examine a potential toxicity of multiwall carbon nanotubes functionalized with carboxyl groups (MWCNTs-COOH) on red blood cells. Using optical microscopy, spectrophotometry and Mössbauer spectroscopy we investigated size and shape changes of erythrocytes, their stability and functionality resulting from MWCNTs-COOH action, respectively.

We observed, that erythrocytes swelled and their shape became irregular in the presence of MWCNT-COOH. Osmotic resistance curves showed variable behaviour and they strongly depended on the applied concentrations of MWCNTs-COOH. This suggests complex interaction of these carbon nanotubes with the membrane structures of red blood cells. Moreover, we found that MWCNTs-COOH could modify the affinity of hemoglobin to bind O2.

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Synthesis and analysis of operational parameters the Nickel Ferrite- Cross-Linked Enzyme Aggregates (NiFe-CLEAs) of glucoamylase and lipase

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Immobilization allows to use enzymes in many industrial branches thanks to the possibility of their repeated use and increase of their stability related with often unfavorable, for delicate biological macromolecules as enzymes, conditions of reactions often used in industrial process. The enzymes' immobilization is threated by many researchers as "an art". In classical approaches its requires use of an appropriate carrier that meets all pre-designed requirements. In the era of searching for environment-friendly technologies and reduction of costs the ideal carriers for enzymes immobilization should be relatively cheap, easily available and biodegradable. Apart popular approaches for enzymes immobilization the Cross-Linked Enzyme Aggregates (CLEA) are alternative that has many advantages. For synthesis of CLEAs is not necessary a support but only suitable method of protein precipitation and crosslinking agent that will not denature an enzymes. The CLEAs can be combined with magnetic field sensitive materials which increases the areas of their application.

The aim of presented study was synthesis of magnetic field sensitive CLEA from commercially available preparations of glucoamylase and lipase with use a nickel ferrite nanoparticles and three phase portioning method for protein precipitation.

The obtained NiFe-CLEAs for both enzymes were characterized with good operational parameters without significant reduction in enzymes catalytic properties compare to its native form. The introduction of magnetic nanoparticles to NiFe-CLEAs allowed for easy operation of immobilized enzymes that markedly simplified using of it.

Multiple sub-lethal antimicrobial photodynamic inactivation and ciprofloxacin treatments lead to S. aureus sensitization to gentamycin and doxycyclin

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Introduction. After almost 100 years after the discovery of the penicillin, future of antibiotic therapy is uncertain. Growing number of multi-drug resistant organisms, especially *Staphylococcus aureus*, forces the alternative therapies development. Photodynamic inactivation (aPDI) is a very promising therapeutic, multi-targeting option which causes functional and morphological damages in bacterial cells. aPDI consists of three elements: photosensitizing agent (PS), appropriate wavelength light and oxygen.

Aim of the study. The current study was aimed to investigate if repeated exposure to rose bengal sub-lethal inactivation, antimicrobial blue light and ciprofloxacin treatments affect susceptibility to different antibiotics.

Materials and methods. Reference strain of *S. aureus* US300 (CA-MRSA) was used in the experiments. Irradiation was performed with two LED light sources that emitted blue (λmax 411 nm) and green (λmax 515 nm) light. 15 repeated cycles of sub-lethal photoinactivation and ciprofloxacin treatment (1/2 MIC) was followed by bacteria re-growth overnight. A potential reduction in susceptibility to different antimicrobials was tested according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST). The MIC values of gentamycin, doxycycline, vancomycin, chloramphenicol and rifampicin was determined by broth microdilution method.

Results. The obtained results demonstrate that multiple sub-lethal phototreatments may lead to *S. aureus* sensitization to antimicrobial agents. Upon 15 consecutive cycles of sub-lethal photoinactivation, the 32- and 128-fold MIC reductions were observed for doxycycline and gentamycin, respectively. The same phenomenon was observed in all three biologically independent repetitions.

Conclusions. aPDI/aBL-induced DNA damage may be responsible for the genetic alterations that lead to the increased susceptibility of *S. aureus* to the antibiotic treatment.

Utilization of oligonucleotide molecular beacon probe on graphene oxide nanocarrier for the detection of survivin mRNA in colorectal cancer cells

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A basic molecular beacon (MB) probe is a single-stranded oligonucleotide with the structure of a hairpin. In this probe, reporter (fluorescent dye) and quencher (Dabcyl) are attached to opposite ends of the strand. In the absence of target, hairpin structure is closed and reporter and quencher are close to each other resulting in fluorescence quenching. In the presence of a complementary target oligonucleotide the probe changes conformation and hybridizes with target separating the dye from quencher and restoring fluorescence [1]. The studies have shown that combination of the MB probes with a graphene oxide (GO) nanosheet carrier seems to be a promising idea for the cancer diagnostics and gene delivery.

In this work, we have investigated the interactions between a graphene oxide (GO) nanosheet nanocarrier and a survivin molecular beacon (SurMB), functionalized by attaching fluorophore Joe and quencher Dabcyl (SurMB-Joe). The mechanism of the hairpin-hairpin interaction of the GO-bound SurMB-Joe with target oligonucleotides has not been tested before. Molecular dynamics simulations of interactions of Joe and Dabcyl with GO carriers have shown the formation of hydrogen bonds. Moreover, *in vitro* studies were performed to detect intracytoplasmic survivin mRNA in SW480 colorectal cancer cells using GO@SurMB-Joe nanoprobes [2].

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Searching for the right model: environmental niche modelling of one bat species

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All species of Ukraine's bat fauna are protected by both governmental laws and international conventions. There is lack of information about preferences in choosing the roosting sites, along with rare presence records of some bat species.

Deepen knowledge about the ecology of bats is very important, as it will help strengthen and improve the conservation methods, as well as protect the real and the potential roosting sites.

In this study, two Natterer's bat Myotis nattereri (Kuhl, 1817) distribution models were built using computer software MaxEnt, which is based on maximum entropy method.

For the first model, database built on all available presence data and free Worldclim version 2 bioclimatic and climatic variables were used. The second model also included MODIS Land Cover 1 (2001) map (IGBP global vegetation classification).

Results show that usage of land cover layers along with climatic variables positively affects the modeling results. According to the first model, two most important factors which affect the model are temperature seasonality (with p.c. — percent contribution — 19,6) and precipitation in April (14,6 p.c.). The second model specifies closeness to deciduous broadleaf forests (33,7 p.c.) and absence of the croplands (13,8 p.c.) as the most informative and important variables, and moves temperature seasonality and April's precipitation to 3d and 4th places correspondingly. Both models' prediction maps show the new potentially suitable areas for Natterer's bat presence and living, which, compared between themselves, differ enough.

As for the conclusion, this result shows the importance of usage of more advanced remote sensing technologies for bat species distribution modelling. This study is a base for the future researches, model improvements and fieldwork verification of the modelling results.

New electrode modules application for cell electroporation

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Electroporation (EP) is a physical method based on applying external electric field to the cells. This method allows to introduce non-permeant and low-permeant molecules into cells. Electroporation is used in anticancer therapy for enhancing drugs transport into cells during electrochemotherapy (ECT). This is a relatively new treatment method that requires further research.

The application new electrode modules for electroporation was studied. Traditionally, electroporation is carried out in the cuvettes on cells in a suspension. To make the testing conditions more similar to those *in vivo*, two electrode modules for electroporation of cells growing in a monolayer were designed and tested. One of them was made of two needle electrodes and the other one consisted of a cylindrical and needle electrode located coaxially. Two human skin cell lines were chosen: normal (HaCaT) and tumor (A375). Two chemotherapeutics were tested: doxorubicin and 5-fluorouracil. Cytotoxicity of drugs was evaluated and the cell viability after electroporation was determined. The effectiveness of introducing the drugs into cells was examined. It was investigated whether the introduced drugs significantly decrease cell viability in comparison to cells which were incubated with the drugs.

The obtained data showed that electroporation with the use of the designed electrodes modules can be used to introduce drugs into cells. Electroporation alone does not significantly reduce cell viability. The effectiveness of drugs penetration into cells depends on their type and cell line. The cell viability after electroporation with 5-fluorouracil was 30-40% lower in comparison to cells viability after incubation (without applying external electric field). In case of doxorubicin, there were no significant differences. The obtained results suggest that the designed electrodes can be successfully applied for introducing drugs into cells, in this way effectiveness of cancer treatment can be improved.

Dynamic kinetic resolution of primary amines using rotating bed reactor

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Due to the chiral nature of all living organisms, there is an increasing demand for enantiomerically pure compounds, e.g. primary amines. Chiral amines constitute synthetic building blocks and key targets in manufacturing of a wide range of chemical products, such as pharmaceuticals, agrochemicals and fragrances. In recent years significant efforts have been dedicated to the enantioselective synthesis, since the most common resolution of racemic mixtures is limited to maximum theoretical yield of 50%. Perhaps, the most promising approach is the dynamic kinetic resolution (DKR) combining enzymatic resolution with *in situ* racemization. The DKR process is a bi-catalytic reaction involving enzyme for the asymmetric transformation and metal complex or nanoparticles for the racemization. Such combination enables complete conversion of the racemic mixture into a single enantiomer. The most challenging issue is matching compatibilities and activities of both catalysts, which optimally operate under different conditions. The research was designed to develop a highly effective rotating bed reactor for DKR protocol to obtain enantiomerically pure primary amines. Studies of the racemization of (*R*)-1-phenylethylamine catalyzed by silica-supported palladium nanoparticles were carried out. Furthermore, the combination of palladium catalysts with Novozym 435 in DKR process was studied.

Acknowledgements
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Fine-tuning of the Z chromosome gene content in lacertids

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Among amniotes, squamate reptiles are especially variable in mechanisms of sex determination, although some lineages possess highly evolutionary stable sex chromosomes. Still very limited knowledge of the genetic content of squamate sex chromosomes precludes a reliable reconstruction of the evolutionary history of sex determination in reptiles and consequently in amniotes. Female heterogamety with a degenerated W chromosome typifies the lizards of the family Lacertidae, the widely distributed Old World clade including several hundreds of species. We selected candidate loci for Z-specific genes from transcriptomes of females of three lacertid species (*Takydromus sexlineatus*, *Lacerta trilineata* and *Darevskia dahli*) and validated them by the comparison of copy numbers between female and male genomes by quantitative PCR. This technique also proved to be a reliable technique for molecular sexing of the studied species. We suggest that this approach is effective for the detection of Z-specific and X-specific genes in lineages with degenerated W, respectively Y chromosomes. The analysed gene content of the Z chromosome revealed that lacertid sex chromosomes are not homologous with those of other reptiles including birds, but instead the genes have orthologs in the X-conserved region shared by viviparous mammals. It is possible that this part of the vertebrate genome was independently co-opted for the function of sex chromosomes in viviparous mammals and lacertids because of its content of genes involved in gonad differentiation.

Microbial modification of pregnenolone by entomopathogenic fungi *Isaraia farinosa*

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Pregnenolone is a neurosteroid which is mainly produced in the gonads, adrenals and the central nervous system. It is synthesise from cholesterol and in the next stages of the metabolic pathway it can be transformed into DHEA, progesterone, testosterone, estrogen, glucocorticoids and mineralocorticoids[1]. Numerous studies shows that pregnenolone can interact with GABA and NMDA receptors which affects the learning and memory processes. Moreover anti-stress, anti-depressant and anti-inflammatory effects were observed[2]. The concentration of this compound in the human body decreases with age.

Isaria farinosa belongs to a specialised group of entomopathogenic fungi which are able to parasitizing insects[3]. This microorganism can be used as an effective biocatalyst due to the high activity of cytochrome P-450 that enables hydroxylation of steroids compounds. Biotransformation of pregnenolone by entomopathogenic fungi has not been described in the literature. In the presented studies we used several strains of *I. farinose* to obtain hydroxy derivatives useful in thetreatment of nervous disorders. The biotransformation process was controlled by GC and TLC techniques, the products' structures were determined by ^{1}H NMR, ^{13}C NMR, and correlation spectroscopy (HMBC, HMQC).

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Resistance phenotypes of *Listeria* spp. strains isolated from soil environments

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The genus *Listeria* (type Firmicutes) comprises of 20 species, including 2 pathogenic – *Listeria monocytogenes* and *Listeria ivanovii*. *L. monocytogenes* is an opportunistic human pathogen causing severe food poisonings, with a high mortality rate. Therefore, most studies on *Listeria* spp. antibiotic, heavy metal and disinfectant resistance focus on strains isolated from clinical cases or from food and food-processing environments. Much less is known about resistance phenotypes of *Listeria* spp. isolated from soil samples. Strains originating from natural and urban soils might be a reservoir of resistance genes. Since many resistance determinants identified so far in *Listeria* spp. were located on plasmids, they can potentially be horizontally transferred among bacteria.

A total of 155 *Listeria* strains (representing 6 species: *L. monocytogenes, L. seeligeri, L. innocua, L. ivanovii, L. welshimeri* and *L. marthii*) were isolated from soil samples of natural and urban environments and tested for susceptibility to eight antimicrobials (ampicillin, meropenem, gentamicin, erythromycin, cotrimoxazol , ciprofloxacin, tetracycline and rifampicin), a disinfectant (benzalkonium chloride) and two heavy metals (cadmium and arsenic). Only one strain (*L. seeligeri* isolated from an urban soil sample) was resistant to a single antibiotic – ciprofloxacin. However, numerous strains showed resistance to cadmium, arsenic and benzalkonium chloride. Resistance determinants were identified based on PCR analysis. Additionally, all isolates were analyzed for the presence of plasmids. 33 of the analyzed strains contained plasmids, and the presence of plasmids correlated with the presence of some variants of resistance genes.

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Detection of the CC398 clonal complex *staphylococcus aureus* isolated from horses

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Admission

There are several known coagulase-positive staphylococci species that are important in veterinary medicine. The best known species is *S. aureus*, which is part of the natural microbiota of the skin and mucous membranes of humans and animals, including horses. The importance of *S. aureus* increased with the emergence of methicillin-resistant strains. MRSA (ang. Methicillin-resistant Staphylococcus aureus). During the last decade, meta-clays resistant to methicillin appeared in the world called *S. aureus* called livestock-associated (LA-MRSA) isolated from livestock, mainly from pigs, cattle, poultry and horses. These strains belong to the ST398 sequential type that forms the clone complex CC398. MRSA ST398 are multi-resistant and have zoonotic potential.

Purpose of research

The aim of the study was to determine the belonging of *staphylococcus aureus* isolated from horses to the clonal complex CC398.

Materials and methods

The research material consisted of 21 strains of *S. aureus*, isolated from horses. Bacteria were grown on a solid Columbia Agar medium with a 5% drop of sheep blood at 37°C for 24 hours. PCR was used to determine the clonal complex. The analysis consisted of two parts. The first PCR reaction was to check whether the isolated *S. aureus* strains belonged to the clonal complex CC398. The second PCR reaction was to check whether *S. aureus* CC398 strains belong to the CC398 complex, but only identified among the strains found in horses.

Results and conclusions

Based on the obtained results, it was found that 90% of the *S. aureus* strains tested belonged to the clone complex CC398. These results indicate that horses in Poland are also a reservoir of staphylococcus aureus representing the clonal complex CC398.

The elusive perpetrators – high-throughput sequencing in search of photos destroyers

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Microorganisms are known for their abilities to adapt to any environmental conditions even the most extreme. It is caused by the occurrence of microbial consortia consisting of various bacterial and fungal species. Metabolic activity of such microbiome may lead to degradation of substances with a stable chemical structure. This process is usually undesirable, especially in case of culture heritage objects, because of their aesthetical changes and loss of mechanical properties. For the correct protection of historical materials, it is necessary to analyze the diversity of microorganisms inhabiting material. The overall characteristic of microbial community structure is possible with analysis of DNA extracted from surface. When it comes to the analysis of valuable and sensitive materials, such as historical photographs, it is crucial to employ non-invasive sampling methods. However, they do not provide a large amount of biological material. Therefore, it is important to select the appropriate DNA extraction protocol. Inappropriate selection of the DNA extraction method may cause incorrect and questionable results of identification of microorganisms.

The research aim was to determine effect of non-invasive sampling methods and DNA isolation protocols on yields and quality of microbial DNA from paper-based objects. Seven different methods were used to extract DNA from 19th century photographs. The concentration and purity of DNA were measured spectrophotometrically and fluorometrically. To assess the suitability of DNA for further high-throughput sequencing, V3-V4 region of bacterial 16S rRNA and fungal ITS region were amplified. Comparison of results showed that yield and quality of DNA depended on DNA extraction protocols as well as sampling method, which indicates the need to optimize both components of the analysis.

The violaxanthin cycle and the greening process in young wheat seedlings

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A violaxanthin (Vx) cycle is a crucial set of biochemical reactions protecting plants from intense illumination. Although this cycle was studied in different experimental setups, little is known about its activity at the very first steps of greening (deetiolation) in young angiosperm seedlings.

In this work we assessed the activity of Vx cycle during the deetiolation of 4-day-old wheat (*Triticum aestivum*). The greening process was initiated by subjecting the seedlings to the white light illumination with the intensity of either 100 or 500 μmoles of photons per m^2 per s for either 1 or 4 hours. Then, the seedlings from all tested conditions were exposed to intense illumination (1500 μmoles of photons per m^2 per s) to initiate the light stress. After that, the seedlings were kept in darkness for 6 hours. The samples for HPLC and fluorescence spectroscopy at 77K analyses were collected during deetiolation, light stress conditions and the following dark treatment.

The contents of Vx cycle pigments and chlorophyll were measured by HPLC. The results were analysed with respect to the assembly of the photosynthetic complexes, measured by fluorescence spectroscopy at 77K.

The activity of the Vx cycle was initially higher in seedlings that were deetiolated for 4 hours, in contrast to those that were deetiolated for only 1 hour. Moreover, the changes of Vx cycle pigment contents during the light stress conditions were less pronounced in long-deetiolated seedlings. Both PSII and PSI peaks were present in fluorescence spectra of long-deetiolated seedlings. PSI peak was not present in spectra of short-deetiolated seedlings.

We concluded that the time of deetiolation, rather than the used intensity of illumination, was more significant for the course of the greening process and for the beneficial activity of the Vx cycle.

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Drosophila melanogaster – an excellent model for studying stress response and its impact on the cell nucleus

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The fruit fly is a great model organism for comprehensive genomic, developmental and molecular studies. It is also a perfect model for investigating stress response and mechanisms accompanying this kind of cellular reactions. In fruit flies heat shock results in the transcription shutdown which affects almost all *loci*, except several special spots – heat shock *loci* – in which transcription gets highly activated. Heat shock induction gives an opportunity to analyze global changes in transcription and other associated mechanisms. There are scientific reports which link transcription shutdown and the rearrangement of chromatin with changes in the nuclear lamina.

Nuclear scaffold consists mostly of lamins – proteins performing a range of important structural and regulatory functions. Lamins are responsible for the shape of the nucleus and are regulating nuclear events such as transcription, DNA replication/repair, and chromatin organization. It is obvious, that in order to play such a variety of functions, lamins have to interact with different nuclear proteins, which are directly responsible for a particular function. Since lamin itself as well as through interacting proteins (e.g. topoisomerases II, HDACs, otefins) is also implied to participate in reaction to stress induction, we decided to analyze their potential role in that processes.

We use fruit flies embryos and cell lines to study the effect of stress on protein and gene expression. We also investigate the subcellular distribution of lamins and interacting proteins. Here we report our results from western blots, RT-PCR analyses, and high-resolution confocal microscopy. We confirmed that although lamin Dm and topoisomerase II expression is not changed as an effect of heat shock, the pattern of the protein phosphorylation is modified. We also show the redistribution of chromatin and immunostaining images for nuclear proteins in normal, heat shock and recovery conditions.

Impact of cold stratification of apple (*Malus domestica* Borkh.) seeds on malondialdehyde (MDA), phenols content and total antioxidant activity in embryonic axes

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Apple seeds (*Malus domestica* Borkh.) are characterized by deep embryonic dormancy. Removal of this type of dormancy requires among others cold stratification (5°C) in moist sand for at least 40 days. The enhancement of reactive oxygen species (ROS) content in apple embryos during cold stratification has been shown.

ROS are known for dualistic nature in cells, depending on concentration. These compounds play a signalling role in different physiological processes including dormancy alleviation following by germination of seeds. This ROS function as regulators occurs only at the low (physiological) concentration placed in the "oxidative window" range. On the other hand, high (pathophysiological) concentration leads to oxidative modifications of proteins or lipids and causes cell damage. Cellular regulation of ROS content is provided by the antioxidant system. Non-enzymatic antioxidant system (e.g. phenols compounds) plays a crucial role in the ROS content regulation during seed dormancy alleviation and during first phases of germination.

In this work, the impact of cold stratification on malondialdehyde (MDA), a product of lipid peroxidation, total phenolic compounds content, and total antioxidant activity in apple embryonic axes were investigated. Apple seeds were cold (5°C) stratified in water moistened sand for 7, 14, 21 and 40 days. After stratification, embryonic axes were isolated from seeds. Cold stratification resulted in decrease of MDA content in apple embryonic axes. Moreover, increase of phenols content and total antioxidant activity were observed.

Effect of a prior experience in the model of socially transferred fear in Wistar rats

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Emotional contagion is one of the most fundamental forms of social interaction, observed across many species. To study this process, we use a rat model of observational fear learning. In our paradigm one animal (Observer) watches a partner (Demonstrator) while the latter receives foot-shocks. Typically Observer rats display vicarious freezing reaction, mimicking the behavior of Demonstrator. The aim of the study was to examine effect of a prior experience of observer rat in observational fear learning paradigm in terms of behavioral and neural activity.

In order to investigate the effect of prior experience we studied two groups of Observers: naíve and experienced. The latter group received mild foot-shocks three days before undergoing social transfer of fear paradigm. We also recorded ultrasonic vocalizations emitted during the social interaction.

After behavioral paradigm, animals were sacrificed and perfused. We collected brains and cutted slices with basolatoral and central amygdala nuclei. Then we performed double immunohistochemistical stainings for two proteins: c-fos (used as a proxy for neural activity) and somatostatine (which is known to be expressed in amygdala neurons which control freezing reaction).

Obtained results let us conclude that prior experience does not affect behavior in fear conditioning. Low cell activity does not allow to clearly determine the neuronal activity in this process. It was confirmed that animals facing imminent danger emit aversive ultrasonic vocalizations at frequency of 22 kHz.

Citrullinated *Porphyromonas gingivalis* surface proteins as a crucial factor enhancing inflammation in human gingival fibroblasts and their differential response

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Porphyromonas gingivalis (*Pg*) is the keystone oral pathogen implicated in development and progression of periodontitis (PD). One of numerous virulence factors of *Pg* is peptidylarginine deiminase (PPAD), an enzyme, which enables this pathogen to citrullinate proteins and peptides, both host- and bacterium-derived. Citrullination of *Pg* surface proteins is crucial for adhesion and invasion of human gingival fibroblasts (HGF) by *Pg*. Previous studies showed that *Pg* can stimulate HGF to express large quantities of proinflammatory cytokines and prostaglandins. Moreover, it was shown that PPAD mutants induced much lower levels of *COX-2* and *mPGES-1* mRNA than the wild-type strains. The aim of this study was the identification of citrullinated protein(s) on the surface of *Pg*, which are responsible for inflammation induction in HGF and comparison of the response in HGF from healthy donors and PD patients. To this end we infected HGF with wild-type ATCC 33277 and sparsely fimbriated W83 *Pg* and mutants of both strains expressing catalytically inactive PPAD ($PPAD^{C351A}$). Using qPCR, we checked the expression level of COX-2 and mPGES-1, the key enzymes in the PGE2 synthesis as well as IL-6, IL-8 and CCL-2. Infections with the wild-type strains induced much higher levels of expression of all examined genes than the PPAD mutants. However, the effect was significantly lower in wt-W83 and the W83 ^{C351A} mutant strain than wt-ATCC and $ATCC^{C351A}$, respectively. We observed similar responses after treatment of HGF with outer membrane vesicles and major fimbriae isolated from both *Pg* strains and their $PPAD^{C351A}$ mutant strains. Worth to emphasize, HGF from PD patients responded stronger to *Pg* infection than healthy donors-derived cells. Cumulatively, we suggest that the vital factors enhancing inflammation are citrullinated fimbriae and epigenetic alternations in HGF.

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One-step purification and kinetic analysis of phospholipase A2 from the venom of *Vipera wagneri*

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Snake venom is a complex mixture of mainly enzymes and non-enzymatic proteins. They can act on various systems leading to cytotoxicity, cardiotoxicity, myotoxicity and neurotoxicity. One of the main neurotoxins present in snake venom are phospholipases A_2 (PLA_2). They catalyze the hydrolysis of 2-sn-phospholipids producing free fatty acids and lysophospholipids. They are low molecular weight proteins (13-19 kDa) requiring Ca^{2+} for catalytic activity and possessing usually 6-7 disulphide bridges, which makes them extremely stable. Apart from their toxic activity, phospholipases A_2 also often show other properties with therapeutic potential. Thus characterization of new PLA_2 from snake venom is a very interesting area of study.

The aim of the project was the purification and kinetic analysis of PLA_2 from the venom of *Vipera wagneri*. Phospholipase A_2 was purified using one-step anion exchange chromatography. Analysis of enzymatic activity of the fractions obtained during chromatography showed the presence of three picks of phospholipase A_2 activity in *Vipera wagneri* venom. The first one did not bind to the column and the two others were eluted from the column with 0.5M and 1M NaCl, respectively. Because of the highest activity we characterized the first, not bounded enzyme. This PLA_2 exhibits the Michaelis-Menten kinetics with Km = 890μM and an optimum pH at 7.5. In the presence of magnesium, barium and manganese ions the catalytic activity did not change significantly compare to calcium ions. However, copper ions acted as strong inhibitor.

Nanosilica from biomass for heavy metal removal

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Plant wastes are being used intensively for the preparation of nanomaterials finding diverse applications in biotechnology. *Equisetum* spp. have an high content of silica naturally deposited in their tissues. The aim of this study was to evaluate the usefulness of silica particles obtained from horsetail (*Equisetum arvense* L.) biomass in dichromate ion adsorption.

Nanosilica was obtained from *E. arvense* L. in a two-step process. First, powdered plant sample was refluxed with HCl solution followed by filtering and washing. Next, dry sample was subject of mild oxidation in air. FT-IR results demonstrated that the main component of the obtained white powder was SiO_2. BET specific surface area of the material, measured by low-temperature nitrogen adsorption was 305 m^2/g.

Dichromate adsorption efficiency was analyzed in function of the amount of used nanomaterial and of ion concentration. A linear increase in total chromate adsorption was observed, with a maximal adsorption around 100 ng/mg of nanomaterial. Cytotoxicity of the obtained material was tested in human microdermal endothelial cells using Alamar blue viability test. Silica material showed a median lethal dose (LD50) equal to 62.5 µg/ml after 24 hours of exposition.

Nowadays, nanomaterials are gaining popularity for their applications in environment safety, for example in the treatment of wastewater. In this context, our study showed that nanosilica obtained from horsetail biomass can offer potential use in environment protection.

The elaboration of an additional melanoma diagnostic procedure based on label-free methods

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Melanoma is the deadliest skin cancer that occurs globally each year in the amount of 132,000 cases. Patients with primary melanoma not spread beyond the skin have a 98% relative survival rate, for those with malignancy spread to the nearby structures or lymph nodes it drops to 64%. In the highly advanced stages (23% relative survival rate), no curative treatment is available, that is why only a surgical intervention at early stages gives the best therapeutic results. The non-invasive dermatoscopic investigation is the first step for melanoma diagnosis, however, it relies on the subjective assessment. Also, histochemical staining of the removed tissue requires highly experienced histopathologist for a proper examination. That is why the elaboration of an additional objective melanoma diagnostic procedure would be beneficial for society.

We have developed a procedure that enables us to objectively distinguish melanoma cells from different progression phases. As it is known on the surface of cells different kinds of glycans are present. The quartz crystal microbalance with dissipation monitoring measurements of specific lectin-glycan interactions can show the degree of malignancy of the analyzed cells through the affinity value and viscoelastic dependency (D(f)) of the created lectin-glycan complexes. For example, cells with a great metastatic potential have both higher affinity for lectin Concanavalin A and large viscoelastic dependency in contrast to cells with low or non-metastatic potential. The method was established on the commercially available human melanoma cell lines (primary and metastatic) and next its proper functioning was checked with melanoma cells isolated from patients. Several cultures of melanoma cells were obtained due to two different isolation procedures and then the cells were confirmed to be melanoma cells by means of gene expression analysis (qPCR).

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Exploiting pangenomes: comparative genomics and deep phenotyping of the plant-associated genus Ensifer

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Rhizobia are biotechnologically relevant bacteria able to form symbiotic interaction with legume plants and perform symbiotic nitrogen fixation (SNF). One of the model genera of rhizobia is the genus Ensifer (syn. Sinorhizobium), where it is possible to find both symbiotic and non-symbiotic species.

The aim of this work was to shed light on the amount of gene sets and biotechnologically-relevant abilities of the symbiotic and non-symbiotic species from genus Ensifer by comparative genomics and Phenotype Microrray™. The long-term aim is the improvement of SNF by genomic manipulation using the Ensifer large pangenome [1].

Comparative genomics identified two distinct clades separating the symbiotic and non-symbiotic species. Genes involved in nodule formation and nitrogen fixation (*nodA, nodB, nodC, nifD, nifK, nifH*) were almost exclusively present in the symbiotic clade. The separation into two groups was also shown by Phenotype Microrray™ analysis. In particular, the non-symbiotic species showed higher resistance to harsh conditions (e.g. heavy metal) compared to the symbiotic species.

These data provide the basis for the application of improved strains, combining such relevant features of the non symbiotic species into the symbiotic ones, toward legume-assisted bioremediation [2].

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Better together. Silver nanoparticles, *Iris pseudacorus* plant extracts and their synergistic interaction act against bacterial human pathogens

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Bacterial resistance to antimicrobials is not a new problem but in face of increasing number of new strains quickly gaining resistance it is an urgent to be solved. To slow down this process there is a need not only to search for new effective compounds but also methods with combined diverse modes of activity. Such combinations may provide a possibility to use differently acting compounds in reduced dose and toxicity but with the same (additivity) or even better (synergism) activity against pathogens. In many cases it may also unlock possibility to use compound to which microorganisms are already resistant. Synergistic combinations of antimicrobials have already been introduced as successful strategies to combat infections, especially those involving multidrug resistant bacteria.

In this studies we were analysing antimicrobial activity of extracts from tissues of Iris pseudacorus plants and their possible positive interactions with commercial silver nanoparticles (AgNPs) against human pathogens.

Antimicrobial activity of methanolic extracts from *I. pseudacorus* rhizomes was tested using the microbroth dilution method against human pathogens, reference strains and clinical isolates, both susceptible and resistant for commercially available antibiotics. Minimal Bactericidal Concentration (MBC) was determined for the extracts against: Staphylococcus aureus, Pseudomonas aeruginosa, *Klebsiella pneumonia, Escherichia coli* and *Enterococcus faecalis*, also for AgNPs against *S. aureus* and *P. aeruginosa*. Analysis of possible interaction between extracts and AgNPs, based on previously established MBC values, was tested by Checkerbord Tritation Method against *S. aureus* and *P. aeruginosa*. Our results revealed antimicrobial activity of *I. pseudacorus* extracts against all tested strains and the synergistic interaction of extracts and AgNPs against *P. aeruginosa*.

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Modulation of daunomycin biological activity by silver nanoparticles

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According to World Health Organization research, cancer is the second leading cause of death in the world [1]. Chemotherapy is one of the most frequently chosen method to fight with cancer. Daunomycin (DAU) is an anthracycline antibiotic routinely used in acute leukemia therapies. Unfortunately, DAU causes a number of side effects, including potentially life-threatening cardiotoxicity, which may appear even after several years [2]. To eliminate or reduce a number of these abnormalities new methods for delivering drugs are developing.

One of the ideas is to use nanoparticles as carriers of the active substance. Such nanoparticles would facilitate the transport and accumulation of a drug at a specific site in the biological system, improve the pharmacodynamic and pharmacokinetic parameters of the drug, which would result in increased antibiotic effectiveness while reducing its toxicity. In my work I used silver nanoparticles (AgNPs), as a potential carrier for antibiotics. Silver has a number of properties that make it a unique metal for research, and due this fact AgNPs are currently the most known and commercialized metal nanoparticles.

This work presents results of multiple biophysical studies (UV-Vis spectrophotometry, fluorescence spectroscopy, dynamic light scattering and isothermal titration calorimetry, mutagenicity tests), which demonstrate direct interactions between AgNPs and DAU. Moreover, based on these results we were able to determine thermodynamical properties of the AgNPs-DAU complexes and assess their role in modulation of biological activity of DAU.

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New way to fight *Staphylococcus aureus* wound infections

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Staphylococcus aureus is a very abundant bacteria responsible for 20-80% of infections worldwide. In Poland over 30% of the population is colonized by *S. aureus*, mainly in upper respiratory tracts and skin surface. Although for healthy individuals the bacteria cause no harm, for people with compromised immune system, like hospitalized patients, become a serious threat. Extensive use of antibiotics over the last few decades resulted in appearance of antibiotic resistant *S. aureus* (MRSA). Skin infections are caused by many species of bacteria, and can occur spontaneously, or in a wound, ulcer or burn. Wound infections are typically caused by *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Proteus*, *Pseudomonas*, *Escherichia* and others. Antimicrobial wound management is a major challenge that continues to require new solutions against microbes and their biofilms. The main goal of antiseptic therapy is to eliminate or at least significantly reduce bacteria in order to prevent infections and reinfections. There are several antibacterial agents combined with wound dressing that are available for treatment, like antibiotics, silver particles, hydrogen peroxide and iodine-based preparation. However they have been reported as not effective (antibiotic) or toxic (silver). There is an urgent need of development of new wound dressings, particularly those which are functionalized with new antimicrobials, like antibacterial lytic enzymes. One of them is Auresine, an engineered peptidoglycan hydrolase which eliminates *Staphylococcus aureus* being particularly vicious against strains that are resistant to antibiotics, such as MRSA, leaving natural microflora unharmed. Auresine cleaves cell walls of a wide range of *Staphylococcus* strains. In presented studies we have focused on application of Auresine as an antistaphylococcal agent in newly generated antimicrobial wound dressings.

The clinical course of *Listeria monocytogenes* meningitis compared to other community-acquired bacterial meningitis

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Introduction

Bacterial meningitis (BM) is a life-threatening infectious disease in which not only subarachnoid space and meninges, but also brain parenchyma is involved in the inflammatory reaction (meningoencephalitis). *Listeria monocytogenes* is a gram-positive bacillus principally spread by contaminated food. The main risk factor for *Listeria* meningitis (LM) is age and immunodeficiency. Due to the resistance of *L. monocytogenes* to third generation cephalosporins (empiric treatment of BM), it is important to distinguish a group of patients with an increased risk of *Listeria* meningitis where the drug of choice is ampicillin.

Aim of the study

The aim of the study was to find differences in symptoms and signs, laboratory tests and comorbidities in order to distinguish irregularities characteristic of LM and to evaluate the results of treatment compared to non-*Listeria* bacterial meningitis (NLBM) patients.

Material and methods

Medical charts of all patients with BM diagnosed in Department of Infectious Diseases for Adults between 2010 and 2017 were analyzed. There were 337 patients with BM divided into two groups of *Listeria monocytogenes* meningitis (Group A; n=24) and non-*Listeria* bacterial meningitis (Group B; n=313). The diagnosis was based on the clinical manifestation, CSF tests, positive cultures or positive direct microscopy. All cases of LM were confirmed microbiologically. Symptoms and signs, incidence of comorbidities, deviations in blood and CSF laboratory tests, treatment results were studied in both groups.

Results

The age range was 17-93 years. Patients from group A were older compared to group B (62 years vs. 57 years, p=0.039). The analysis showed no significant differences in symptoms and signs. Patients with LM were more likely to have tumors (29.17% vs. 8.59%, p=0.002) and more often had any immunodeficiency (45.83% vs. 10.58%, p<0.05). Laboratory tests showed a lower WBC level in blood (10.7 cells/mm^3 vs 15. 5 cells/mm^3, p=0.0036), lower granulocytes% (62% vs. 90%, p=0.002) and lower CRP level (150 mg/L vs 230 mg/L, p=0,02) in group A . The CSF tests showed a lower cell count (531.5 cells/mL vs. 1230 cells/mL, p=0.01) and a lower chloride level (113 mmol/L vs. 117 mmol/L, p=0.009) in Group A.

Conclusions

Meningitis due to *L. monocytogenes* is a disease that occurs more often among immunocompromised and elderly individuals. Symptoms and signs are similar in both groups. Patients with LM have a lower cytosis in CSF and a lower WBC level in peripheral blood morphology.

Biological properties of flavanone and its hydroxy derivatives

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In nature, the precursor in the formation of flavonoid subclasses is flavanone whose enzymatic modifications including reduction, oxidation and hydroxylation reactions lead to different flavonoid types.

Flavanones play a significant role in the prevention of many civilization diseases such as cardiovascular diseases or cancer.

Nowadays, many studies focus on the design of compounds that have the potential for binding to DNA.

DNA is the primary intracellular target of an anticancer compound because the interaction between these molecules can block the division of cancer cells and resulting in cell death.

The present work is devoted to reveal several biological actions of flavanones – flavanone, 2'-hydroxyflavanone, 6-hydroxyflavanone and 7-hydroxyflavanone by spectroscopy UV-Vis. UV–Vis electron absorption spectroscopy were applied to find a clue about DNA binding with flavanones.

Additionally, obtaining information about correlation between the structure of compound and its biological activity is very important and helpful in understanding the mechanisms of their interactions, which in turn will facilitate the receipt of potential substances with the desired pharmacological effect.

Results showed a certain relationship between flavonoids and DNA. The best flavanone is 2'-hydroxyflavanone.

Functionalized silver nanoparticles interactions with model mutagen ICR-191

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Currently, nanotechnology and nanomaterials have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices. Among other nanostructures, metal-based nanoparticles, such as silver nanoparticles (Ag-NPs), have attracted increasing attention due to their unique physical, chemical and biological properties. Ag-NPs are able to directly interact with active molecules, what allows them to act as potential drug carriers. Moreover, nanoparticles surface modification is one of the proposed methods for nanoparticles biological activities improvement.

In this study we synthesized silver nanoparticles and functionalized their surfaces with 11-mercaptoundecanoic acid (MUA) and thiobarbituricacid (TBA) residues. Subsequently, we described physicochemical properties of newly obtained Ag-NPs using a wide range of biophysical methods: UV-Vis spectrophotometry, fluorescence spectroscopy, dynamic light scattering and isothermal titration calorimetry. Afterwards, we assessed Ag-NPs ability to form heteroaggregates with model acridine mutagen ICR-191. Next, we examined the potential effect of Ag-NPs surface modifications on AgNPs mutagenicity and toxicity using bacterial reverse mutation assay, called Ames test.

We observed that the modifications in AgNPs surfaces affect nanoparticles ability to self-aggregate, as well as to interact with ICR-191 molecules by forming heteroaggregates. As confirmed by our study, AgNPs surface functionalization is a powerful tool, which can control nanostructures interactions with biological systems. Nevertheless, further efforts should be undertaken to understand surface coating-dependent toxicity.

Effect of pasteurization on melatonin concentration in breast milk

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Human breast milk has many functions, primarily protects the child's body on many levels by stimulating the innate and adaptive immunity, as well as anti-inflammatory, antibacterial and antioxidant. The components of breast milk are proteins, carbohydrates, triacylglycerols, minerals, vitamins, enzymes, hormones, growth factors, blood components and elements of the immune system.

The material for the study consisted of 10 samples of breast milk from the Human Milk Bank, Ludwik Rydygier Provincial Polyclinical Hospital in Torun before and after pasteurization. The pasteurization process took place at 63°C for 30 min. Melatonin concentration was determined in the samples by ELISA (Melatonin ELISA Kit, IBL INTERNATIONAL) according to the manufacturer's procedure.

The concentration of melatonin in breast milk before pasteurization was 0.0-18,03 pg/ml (Me = 2,179, IQR = 8,7), whereas after pasteurization it was 0.0-18,23 pg/ml (Me = 3,227, IQR = 9,255). Differences in the values of the parameter determined were not statistically significant (p = 0.074).

Pasteurization does not affect the concentration of melatonin in breast milk. This is very important for the Female Milk Banks, as they are recommended as centers for the storage of female milk. Melatonin is a very important hormone that, in addition to the regulation of sleep and wakefulness can also affect the digestive system and prevent the formation of collections in children. The information about the lack of pasteurization effect on the degradation of this hormone is therefore of great importance.

The structural-functional analysis of complexes of plasmid replication initiation protein, Rep, and the ssDNA DUE

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The crucial step of the replication initiation of iteron plasmids is the recognition of *origin* sequence by replication protein, Rep. The binding of the Rep protein to the double-stranded DNA (dsDNA) of iterons within the *origin* sequence causes local destabilization within DNA Unwinding Element (DUE) region and its melting. The DnaB helicase is loaded onto exposed single-stranded DNA and next the replisome is assembled to synthesize DNA. The data concerning DnaA protein, the bacterial chromosome replication initiator, revealed that it binds to the specific sequence of dsDNA (DnaA-boxes) via DBD domain (DNA binding domain) and single-stranded DNA (ssDNA) within DUE region via AAA+ domain (ATPases Associated with diverse Activities). Rep protein instead of DBD and AAA+ domains possess Winged Helix domains (WH), which specifically bind to dsDNA. Our data showed that Rep proteins are able to interact also with ssDNA within DUE region. Base on crystallographic data as well as bioinformatic and mass spectrometry analysis, we identified amino acid residues of Rep proteins, which may be responsible for the interaction with ssDNA within DUE region. We constructed and purified Rep proteins variants which were further analyzed for ssDNA binding. Surface Plasmon Resonance (SPR) and Electrophoretic Mobility Shift Assay allowed us to confirm that proposed amino acids are crucial for such an interaction.

Multi-compartment hydrogel capsules for topological 3D co-culture studies

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In this study, we demonstrate new microfluidic methods of precise, reproducible production of compact 3D structures from hydrogel microbeads. We use microfluidics both to formulate hydrogel microbeads as well as to reaggregate them into small close-packed agglomerates of well-defined topology. We propose that such advanced topological structures could be used as new 3D culture scaffolds in which cells get segregated in separate microcompartments to reproduce the morphology and functionality of actual tissues.

Depending on the applied flow rates, we were able to encapsulate from N=2 up to N=12 gelatin beads-cores in a single gelatin shell. The structures had reproducible, predictable topology following the topologies observed earlier for colloidal clusters or in double-emulsion droplets with multiple cores. We also managed to reproducibly encapsulate given numbers of different core types (labelled with TRITC vs FITC). In order to demonstrate suitability for cell encapsulation, we generated simple cell-laden beads (C2C12, fibroblasts, HUVEC) as well as co-encapsulated beads loaded with cancer cells, HUVECs and/or fibroblasts into agglomerates for topological 3D co-culture studies. We found that the re-encapsulation method is suitable to any kind of enzymatically cross-linkable hydrogel, i.e., also to fibrin, typically applied as extracellular matrix substitute, e.g., in angiogenic assays.

This study provides new type of hydrogel microparticles built of several compartments arranged in well-defined close-packed topology. Such particles allow for formation of microtissues with direct cell-cell communication and predesigned spatial arrangement. We are currently investigating suitability of such platform to co-culturing tumor cells with ECs and fibroblasts with the ultimate goal of mimicking cancer microenvironment for studying drug efficacy and tumor development.

Screening of microscopic fungi strains for the production of biosurfactants

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Chemical surfactants such as *SLS* are well known for their ability to lower water tension and to create foam. Those properties are used mostly in personal care products and cleaning agents. However, *SLS* has also drawbacks i.e. it causes skin irritation and degrades slowly. In contrary, biosurfactants combine not only qualitative physiochemical and non-irritant features, but also ecological aspects. They can be obtained from plants, bacteria or fungi.

The aim of this study was to screen the microscopic fungi collected at Faculty of Chemistry, Warsaw University of Technology for strains capable of producing extracellular biosurfactants. The initial characterisation of obtained bioproducts was also performed.

Screening test 'oil spreading' was conducted on thirty strains and eleven of them gave positive effects. Interestingly, seven of them were assigned to *Aureobasidium pullulans* species. Then, three strains WUT62 (*A. pullulans*), WUT128 (*Rhodotorula glutinis*) and WUT165 (*R. babjevae*), which acquired the best results, were cultivated in the medium containing 10% glucose for 96 h at 25°C. The biosurfactants were isolated from the cell-free supernatants by means of extraction with chloroform: methanol 2:1 (v/v) as an organic phase. Analyses including emulsification activity (*%EA* and E_{24}), critical micelle concentration (*CMC*), surface tension and mass spectrometry (*MS*) were carried out. The product isolated from the culture of WUT165 strain scored similarly to chemical surfactants in those experiments.

In conclusion, yeasts from *Aureobasidium* and *Rhodotorula* genres were the most promising producers of biosurfactants under tested conditions. The biosurfactants obtained from the WUT165 culture need further examination because they may have utility in the industrial applications.

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Plant histone deacetylases mutants obtained with CRISPR-Cas9

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Histone acetyltransferases (HATs) and deacetylases (HDACs) are enzymes that modify availability of DNA affecting DNA-dependent processes (gene expression, replication, DNA repair). HATs transfer acetyl groups to lysine residues on histones, which leads to neutralization of positive charge and relaxation of chromatin. This event grants an opportunity for transcription factors and chromatin remodelling complexes to act on the DNA. HDACs, on the other hand, erase acetyl marks from histone tails, resulting in more condensed chromatin. In consequence histone acetylation is usually associated with upregulation of gene expression, in contrary to histone deacetylation which is connected with transcriptional repression.

The aim of this research was to generate *Arabidopsis thaliana* mutants of HDACs with use of CRISPR-Cas9 genome editing system. There are 18 putative HDACs in *A. thaliana*, which are grouped in three superfamilies, called RPD3-like, sirtuins and plant specific HD-tuins. They are responsible for many processes, such as germination, leaf development, flowering induction and embryogenesis. We have been able to obtain lines of deletional *null* mutants for 14 HDACs. It will allow us to describe previously not studied histone deacetylases.

Newly obtained lines will help in understanding the intergenic interactions between HATs and HDACs. With those materials, we also are going to investigate dependency of meiotic recombination on histone acetylation. Those information would be used to develop novel selective breeding strategies of crops and other widely-used plants.

Antioxidant properties of plants of the genus Lycium

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The popularity of natural foods and medical plants which can prevent or inhibit the development of many diseases is growing rapidly nowadays. Recently, interest in the fruit of *Lycium barbarum* L. and *Lycium chinense* Mill., known as Goji berries, has also increased. These fruits, due to the content of nutrients and bioactive ingredients, are classified as superfoods.

The aim of the research was to estimate the antioxidant properties of Goji fruits of different origin. In this study 5 preparations were examined: powdered Goji berry juice (country of origin United States of America), dried Goji berries (China), freeze-dried Goji berries (Poland), natural-dried Goji berries (China) and freeze-dried Goji berries (China). The extracts were obtained by grinding fruits and next mixing them with 70% ethanol or water and using ultrasound-assisted extraction. In the last step, the samples were centrifuged. Antioxidant properties were determined by the mean of radical scavenging capacity assay using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The formation of the free radical $ABTS^{\bullet+}$ was monitored by the measurement of absorbance. Then the results were converted against TEAC-Trolox® Equivalent Antioxidant Capacity.

The results of the study indicate that Goji berries have antioxidant properties and, therefore, its consumption can bring benefits to human health.

The influence of gastrointestinal digestion on metallic nanoparticles of plant origin

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Nanoparticles (NPs) are currently widely used in various branches of industry, agriculture, as well as medicine and scientific research. Such a variety of applications implies nanoparticles' release to the environment during not only their use itself but also production, transport and disposal. As soon as they occur in the soil, nanoparticles may be internalized in plants' roots and subsequently transformed or transported to the upper organs. Mechanisms of nanoparticles uptake and translocation in plants are not yet entirely known. After being taken up by plants, nanoparticles may enter the food chain and eventually human digestive tract. Current knowledge on whether nanoparticles are prone to be absorbed by other organs, which might have consequences for human health, is limited.

The aim of the investigation was to assess the chosen metals uptake to radish cultivated in media rich in CeO_2 NPs as well as their speciation and bioaccessibility during the *in vitro* gastrointestinal digestion simulation. After cultivation in the environment enriched in NPs cerium, which in normal conditions is present only in undetectably low amounts, has been found in all organs of the radish. Less cerium has entered the shoots than the roots. The nanoparticles presence in the environment caused increased uptake of other metals to radish tissues. After the *in vitro* simulation of gastrointestinal digestion, in which pepsin and pancreatin have been used, the analyses of digestive extracts have been conducted, what allowed assessment of plant deriving cerium. Cerium resulted to be easily accessible-the majority of the metal content in plant enters the digestive extracts and possibly is able to be absorbed by human organism. Since cerium dioxide nanoparticles were confirmed to be able to enter the human organism, it seems questionable whether their further use in the industry is safe for human health.

Adropin stimulates ERK1/2 and AKT phosphorylation in 3T3-L1 preadipocytes

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The prevalence of obesity is a serious health and economical problem. Obese individuals suffer from numerous diseases such as type 2 diabetes, cardiovascular diseases and have higher risk of cancer. So far there are not many effective medicines for obesity. More studies are needed to find new potential therapeutic tools. There is evidence that some peptides involved in adipocytes biology may be taken into consideration. Our studies are focused on adropin. Adropin consist of 76 amino acids and is encoded by Energy Homeostasis Associated (*Enho*) gene. Protein sequence of adropin in humans and rodents is identical. Based on animal studies there was found that adropin has an impact on lipid metabolism regulation. Adropin deficiency is associated with increased adiposity. Some data indicated that adropin applications causes stimulation of AKT and ERK1/2 phosphorylation in endothelial cells. Importantly, these protein kinases are also implicated in adipocytes functions. Our previous studies showed that adropin stimulates 3T3-L1 preadipocytes proliferation. Potential mechanism involved in this process was unknown. In the present study we found that adropin stimulates ERK1/2 as well as AKT phosphorylation in 3T3-L1 cells. Phosphorylated, and total ERK1/2, and AKT were detected by Western blot technique. In the presence of pharmacological MEK1/2-dependent blocker of ERK1/2 adropin-induced 3T3-L1 cell proliferation was attenuated. PI3/AKT blocker also partially suppressed basal cell growth. It is known, that AKT plays a role in white and brown adipogenesis. Stimuli of AKT phosphorylation enhance growth and browning of white adipocytes. Obtained data indicates that adropin may be involved in controlling white adipogenesis. In summary, we found that adropin promotes preadipocytes growth via ERK1/2- and AKT-dependent mechanisms.

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Glucose biosensor based on the hybrid microplatform and its comparison with other testing techniques on commercial food-samples

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Biosensor is a kind of chemical sensors consist of sensitive element like enzymes, genes, antibodies etc. which they are able to control the interaction between the substance and the sensitive layer and processing element which it generates signal. Novel innovative materials for glucose biosensor have gained enormous attention due to the need for a cheap and effective blood glucose monitoring. Due to high selectivity enabling their use in many areas like enviromental protection, medicine, food and drug industries etc. Determination of glucose level is one of the most popular and important assay in various media like blood plasma, drugs, juices or dietary supplements. Despite the presence of commercial glucose biodetectors in the market, there are still some limitations in accuracy and errors like aging, manufacturing variances, storage, temperature, coding or proper data calculation. In this work, we discuss the measurement of glucose in real samples using techniques such as photometric assay, glucometers, and using a proposed biosensor's system. Biosensor platforms based on the novel sophisticated magnetite/lignin/polydopamine-glucose oxidase hybrid with the addition of ferrocene and a dedicated carbon paste electrode (CPE/Fe_3O_4/Lig/PDA/GOx/Fc) were fabricated for glucose detection in real, commercially available glucose-based samples. The proposed platform has interesting features like improved thermal and mechanical stability, excellent adhesion for inorganic and organic materials, transferability of electrons and photothermal properties. Greater material stability and durability and the extension of its attractiveness, compared to the current commercial products. The proposed biosensing microplatform has promising features and applicability as a useful biodetector in the food industry.

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Disposable tissues with silver-copper nanoparticles addition used in dairy cows milking routine for mastitis prevention

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Different pathogen species involved in inflammation process in cows udder (mastitis) are frequently resistant for conventional treatment. Therefore, scientists are looking for new and innovative solutions in mastitis prevention. Nanoparticles are becoming one of the most promising agents that are already used e.g., in veterinary or human medicine.

The aim of the study was to estimate if silver-copper nanoparticles addition (AgCu) in pre-milking disinfectant disposable tissues can reduce the number of bacteria existing on dairy cows' teats.

Commercially available disposable tissues without any disinfectant agents were used in the experiment. Tissues were placed in AgCu nanoparticles solution 24 hours before the experiment. The experiment was conducted in dairy herd located in Mazovian voivodeship and maintaining 20 dairy cows of Polish Holstein-Friesian breed. Two teats from each cow were treated as control group and two teats were classified as experimental group. Disposable tissues without AgCu were used to clean teats in control group and placed in sterile cups containing 40 ml of 0,1% NaCl. Tissues with AgCu addition were used to clean teats in experimental group. In the next step disposable tissues without AgCu were used to estimate changes in the number of bacteria in experimental group and were put in sterile cups with NaCl. Microbiological analysis were conducted after 12 hours.

In over 97% of samples was diagnosed at least one pathogen species. Obtained results revealed that disposable tissues with AgCu can decrease the number of bacteria existing on cows' teats. The most frequently isolated bacteria were streptococccci and stapgylococci.

Observed changes in the study suggest that prepared tissues and mixtures could be used in dairy cows milking routine. Therefore, nanoparticles can be useful tool in mastitis prevention. However, further analysis are necessary.

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Cultivation of bacterial endophytes isolated from different wheat varieties and testing their ability to produce cellulases

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Endophytes are microorganisms that use their colonization ability to colonize plant tissues without causing negative effects on them. They can help the plant in growth process or with the fight against phytopathogens.

The aim of the studies was isolation and cultivation of endophytes from two wheat varieties (*Triticum aestivum* L. 'Hondia'; *T. spelta* L. 'Rokosz'), as well as demonstration of their biotechnological potential.

The isolation of endophytes was carried out from wheat plants in the BBCH 13 growth stage. The macerated plant fragments were plated on nutrient agar (7 days, 28°C) after surface sterilization. The morphologically different colonies were passaged to obtain pure strains that then were analyzed for cell wall construction using Gram staining. In the next step, amplifications of DNA fragments were made using primers 27F and 518R, as well as with using DNA isolated from the obtained strains (Sambrook and Russel, 2001). The PCR products were sequenced, followed by bioinformatic analysis of the sequences. The final stage were the analysis of biotechnological properties of isolates using the carboxymethylcellulose test (CMC test, Kasana et al., 2008). The aim of this test was to demonstrate the ability of isolates to produce cellulases.

In total, 122 pure strains were obtained as a result of passaging. Among of studied strains we determined the phylogenetic affiliation of most of them to the genus Bacillus. Largest group showed the Gram+ cell wall structure, some of them were Gram-variability, and individual strains were classified to Gram- bacteria. The CMC test showed that among studied wheat endophytes, there are three groups of bacteria due to the intensity of their cellulolytic activity: low, moderate and highest.

The analysis confirmed assumed aim, that endophytes colonizing wheat may have significant biotechnology potential. The studies with microorganisms inhabiting inner plants are important, especially in food industry.

Analysis of thermal stability of bacteriophages T4 and MS2

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Bacterial viruses called bacteriophages are considered to be a remedy for bacterial infections. They are also regarded as as a model of a eucariotic viruses. In order to use viruses as a drug we needto transport them in low temperatures. Otherwise they will loose effectiveness to infect bacteria. We decided to analyze the thermal profile of two representative bacteriophages i.e.: T4 and MS2.In order to do so we used in silico method of molecular dynamics. Such an approach seems to bebeneficial, because we are able to analyze singe fluctuation of amino acids in atomic resolution.

The bacteriophage T4 is a virus devoted to infect bacteria *Escherichia coli*. Its structure consistsof a head containing genetic information, collar and a tail. The high pressure inside the head is thedriveing force to inject stored genomic material into bacteria. That is why T4 head seems to be mostlyinfluenced by temperature. We focused on proteins of these compartment. The T4 head consist of fourdifferent proteins: GP23, GP24, SOC and HOC.

The bacteriphage MS2 is also a natural enemy of bacteria *E. coli*. Its build is rather moresymetrical. It consists of just one type of protein but in 180 copies. Virus MS2 is a closer relativeto eucariotic viruses regarding build than T4.

Having that in mind we analyzed 200 ns simulations of those proteins, pinpointing changesin secondary structures, root square of deviation and root square of fluctuation in differenttemperatures. Such simulations were made with explicit water model. We were able to observe thermalchanges in MS2 building trimer as well as T4's SOC protein. Running molecular dynamics simulationin temperatures ranging from 300K to 450K, provided us with a necessary data to pinpoint a criticaltemperatures. The amino acids groups of crucial influence of bacteriophages thermal structuralresistance were also identified. Furthermore, we concluded a melting temperatures of the selectedproteins.

Determining the population of endophytes inhibiting *Triticum aestivum* L. 'Hondia' and *Triticum spelta* L. 'Rokosz'

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Endophytes are a group of organisms inhabiting the interior of plant cells, often living with them in symbiosis. Some of their species produce compounds that affect vitality of plants, eliminate pathogens or protect the plant against unfavorable compounds. In order to obtain the DNA of endophytic bacteria, fragments of plants: *Triticum aestivum* L. 'Hondia' and *Triticum spelta* L. 'Rokosz' were surface sterilized, macerated and performed isolation of the genetic material by the PowerSoil DNA Isolation Kit. Received genetic material was amplified using primers 27F and 518R, then electrophoretically screened. Next-generation sequencing was used (Illumina platform). NGS allowed to determine taxonomic classification and the number of taxonomic units. A total of 160,906 readings of examined fragments of 'Hondia' wheat plants were obtained, whereas 137,333 readings were obtained from 'Rokosz'. Wheat microbiome 'Rokosz' was defined by the dominance of species belonging to the genus *Flavobacterium* belonging to the order *Flavobacteriales*, both in the root 8747 OTU (operative taxonomic unit), leaf 20788 OTU and sheath 57720 OTU. The 'Rokosz' wheat microflora was defined by the highest number of operational taxonomic units characteristic to the *Pseudomonas* genus, belonging to the *Actinomycetales* order, the OTU number was respectively 17618 in the in root, 21937 OTU in leaf and 25374 OTU in sheath. The largest population in the grain was the genus: for wheat 'Rokosz' - *Pantoea* constituting 79.33% of the population of bacteria, for 'Hondia' wheat - *Pseudomonas* in the amount of 58.82%. The embryo microbiome was most commonly populated by: the genus *Acinetobacter* in the amount of 47.06% for 'Hondia', the genus *Pseudomonas* 58.82% for 'Rokosz'. Performed research may contribute to determining the biotechnological potential of endophytic bacteria and their application in many areas

Functionalized silver nanoparticles and their interactions with anthracycline antibiotic - doxorubicin

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Nowadays, due to intensive nanotechnology development, various types of nanomaterials are extensively used in many branches of medicine e.g. fullerenes, micelles, liposomes and metallic nanoparticles namely platinum, gold and silver. However, among those nanoparticles, silver nanoparticles (AgNPs) are the most popular. Their application for various medical purposes, such as optical biosensors in diagnostics of scalp and neck cancer or enhancing the antibacterial effect of drugs owe to their beneficial properties, including wide availability, stability and functionality, which distinguishes them from the ionic silver, widely known in pharmacology.

Also an interesting aspect seems to be using AgNPs as templates for surface modifications and as potential carriers for anticancer drugs in the so-called combination chemotherapy. This method facilitates transport, of often unstable compounds, directly to the place of its action. At the same time, they may improve the pharmacokinetic parameters of the drug itself by, for example, increasing its half-life, as well as reducing its toxicity and the amount necessary in order to achieve a therapeutic effect.

Despite the wide range of possible applications of silver nanoparticles, it is necessary to understand the mechanisms of their interactions with drugs and to understand how they can modulate their activity. In this work, functionalized silver nanoparticles were used. Thiobarbituric acid or 11-mercaptoundecanoic acid residues were attached to their surface to improve their biological activitity. We conducted biophysical research (UV-Vis spectrophotometry, fluorescence spectroscopy, dynamic light scattering and isothermal titration calorimetry, mutagenicity tests) on functionalized silver nanoparticles and their interactions with anthracycline antibiotic - doxorubicin.

Immobilized enzymatic cascade for the continuous flow trehalose production

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Trehalose is a dissacharide with unique physical and chemical properties, and hence an important ingredient of various food, cosmetic and pharmaceutical recipes. The growing interests in its application and an expanding market, fuel the search for the effective environmentally friendly method of trehalose production. [1-3]

Herein, we propose a simplified enzymatic coupling cascade, based on two enzymes–thermostableTrehalose Transferase from *Thermoproteus tenax*, and UDP-glucose pyrophosphorylasefrom *Thermocrispum agreste*. A direct application of the free enzymes appeared, however, to be obstructed by a short operational stability, recovery-reusability issues and shelf life.

To overcome these drawbacks we studied a covalent immobilization of both enzymes on to silica monolithic microreactors to propose a novel continuous flow trehalose production system.

The performed experiments showed that immobilization of the enzymes on separate monoliths proved to be better than co-immobilization on to a single microreactor. The thus obtained cascade of the immobilized enzymatic microreactors enables to obtain good yields in a quite short time, and more importantly, excellent process stability for more than 100 hours of continuous operation.

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The most frequent mastitis pathogens occurring in small polish dairy herds

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Udder inflammations (mastitis) are one of the most crucial issues for dairy milk producers. Mastitis generates additional cost for producers and has negative impact on milk technological value. Over 90% of mastitis cases is caused by bacteria. However, excessive antibiotics use influenced on formation of strains resistant for conventional therapy.

The aim of the study was preliminary estimation of the most frequent mastitis pathogens occurring in small Polish dairy herds.

Milk samples were taken from herds (n=23) in which occurred mastitis cases. Milk was placed in sterile cups during evening milking and delivered to Cattle Breeding Division laboratory. Samples were store for 12 hours in the fridge (4°C). After 12 hours milk was used in microbiological analysis. Several specific microbiological mediums were used to diagnose bacterial and fungi pathogens species. In the experiment were used several: Mannitol Salt Lab Agar, Edwards Lab Agar, Rose Bengal, Enterococcus Confirmatory Lab Agar, Pseudomonas CN Lab Agar Base, Eijkman Lactose Medium and Salmonella Shigella Lab Agar medium (Biocorp, Poland). Inoculations were stored for 24-48 hours (37°C, 5% CO_2) to properly diagnose mastitis pathogens.

At least one pathogen species was diagnosed in over 90% of milk samples. The most frequently isolated pathogens were: *Escherichia coli* (76,61%) *Staphylococcus aureus* (41,29%), *Streptococcus dysgalactiae* (40,81%), *Enterococcus* sp. (40,57%) and *Staphylococcus epidermidids* (28,16%). In 7,88% samples were diagnosed fungi from 2 different genera.

Obtained results suggest that staphylococci and streptococci are the main reason of udder inflammations in dairy cows. However, different species from *Enterobacteriaceae* family were diagnosed which may be the reason of poor environmental conditions in dairy herds.

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Chemical modulation of phage stability

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The development of agents for stabilization of bacteriophages will have positive impact on vaccination programme in underdeveloped countries or biocontrol methods in biotechnology industry. Moreover, phages might be drug of last resort in number of cases induced by antibiotic resistant bacteria. It is noteworthy, that some species of bacteriophages are very similar to human viruses, and might be used as their biological models e.g. MS2 is the model of *Rihnoviruses* spp. While the large-scale of phage stabilization projects are based on genetic modifications of bacteriophages genome, in our studies, we focused on chemical methods of stabilization, by using special synthesized molecules as well as natural compounds.

Four bacteriophages, i.e. T4 (*Myoviridae*), T7 (*Podoviridae*), MS2 (*Leviviridae*) and M13 (*Inoviridae*) were tested for their thermal stability. For every temperature (37, 40, 50, 55, 60, 65, 70℃) the decrease of active phages was determined. Physiologically, we observe that even slightly increase of temperature may cause destabilization of capsid structure, as proteins are held together by non-covalent interactions, easy to dissect. Furthermore, the biological and physiological variety of bacteriophages contributed to the differences in the ratio of phage concentration decrease (e.g. T4 phages were able to survive in 50℃ for more than 2 weeks when MS2 phages were vanishing after 48 hours). Depending on phages, an optimal condition for an effective application are different, and the effective and fast method for thermal stabilization is eligible. We studied PEG chain flanked with two intercalator domains which can bind to phage DNA, which empowered whole capsid. It turned out that after addition of our synthesized compound - APEG (acridinyl poly (ethylene-glycol)) - the stability of phages increased significantly. The amount of APEG was very low (3 ng/ml), which makes the method widely applicable. Our method is faster and cheaper than used to date.

Estimation of cytotoxicity of cinnamon oil on breast cancer cell line

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In recent years, there has been an increase in interest in natural anticancer substances contained in plants that would be an alternative to synthetic cytostatics commonly used in cancer chemotherapy. One such substance is cinnamon commonly used and known as a spice in the food industry. It is known for its pro-health properties since ancient times. This plant has strong antioxidant, antibacterial, antipyretic and anti-inflammatory properties that play an important role in human medicine. In addition, its anti-cancer activity is also proven.

The aim of the study was to assess the potential of cinnamon oil obtained from Cinnamon leaves against the cancer cell line MCF-7 adenocarcinoma. 24-hour cell cultures were incubated for 24 hours with cinnamon oil, followed by cell activity and viability assays (NRU and CCK-8). Cell morphology was also performed.

The studies showed cytotoxic properties of cinnamon oil against breast cancer cells in the range of all tested concentrations. These results reflect the morphological examination of cells, which may indicate the anti-cancer potential of cinnamon oil. Moreover, the results indicate a stronger anti-cancer effect of cinnamon oil against the MCF-7 cell line after 24 hours of incubation with the test agent from doxorubicin commonly used in the treatment of breast cancer.

Nonsteroidal selective androgen receptor modulator Enobosarm affects lipid metabolism in isolated rat adipocytes

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Androgens and androgen receptor (AR) affect the metabolism of adipose tissue. The natural decrease in androgen secretion associated with age causes the excessive deposition of visceral fat. Hormone replacement therapy uses synthetic derivatives testosterone that causes side effects. Selective androgen receptor modulators (SARM) are a novel class of AR ligands and show anabolic effect in skeletal muscle. Enobosarm (Ostarine, GTx024) is one of the non-steroidal SARM. The aim of this study was to investigate the effect of ostarine on lipid metabolism using in vitro methods . Additionally, the genomic activity of GTx24 was determine. The intensity of lipolysis and lipogenesis in mature rat adipocytes was measured by incubating the cells in KRB in the presence of ostarine (0.001, 0.01, 0.1, 1, and 10 μM) for 120 and 480 min. Moreover, testosterone was added into the incubation medium as a natural ligand for AR and adipocytes incubated with isoproterenol was a positive control. Additionally, to check the effects of ostarine on AR adipocytes were pre-incubated with AR inhibitors - flutamide and cyproterone acetate. We showed that ostarine in concentrations 0.001, 0.01 and 10 μM decreases the intensity of lipogenesis. Moreover, we noted that enobosarm had a stimulatory effect on lipolysis in concentration of 1 μM after 2 h of incubation as well as in concentration of 0.1 and 1 μM after 8 h of incubation. Ostarine has also a genomic effect on AR. Ostarine is one of the SARMs tested for the treatment of muscle loss. Our research shows that it has a significant impact on lipid metabolism. This indicates the need for further research on selective androgen receptor ligands.

The effect of Qo site inhibitors of cytochrome bc1 on slow- and fast-relaxing forms of semiquinone generated in Qi site

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Cytochrome bc_1, also known as mitochondrial Complex III, catalyzes the reaction of electron transfer between membranous quinone pool and cytochrome c, located in the intermembrane space. During the catalytic cycle, called the Q-cycle, the electrons that are released through the oxidation of quinol at Q_o site are transferred via two separated cofactor chains – high-potential *c*-chain (FeS cluster and heme c_1) and low-potential *b*-chain (hemes b_L and b_H). Consequently, to fully reduce quinone at Q_i site, subsequent oxidation of two quinols at the Q_o site is required. One-electron reduction of quinone at the Q_i site leads to a formation of stable semiquinone intermediate (SQ_i). This phenomenon has been observed for many years. Recent studies have shown that, in fact, there are two forms of SQ_i differing in magnetic properties – the fast- and slow-relaxing SQ_i (SQ_{iF} and SQ_{iS}, respectively). SQ_{iF} is associated with oxidized heme b_H, while SQ_{iS} is present when heme b_H is reduced. Experimentally, SQ_i can be generated by a blockage of Q_o site and an addition of synthetic quinone. In this work, I compared the effect of seven different inhibitors of Q_o site on the generation of SQ_i. I observed that one of them – azoxystrobin increases the amount of slow-relaxing form of SQ_i in comparison to other inhibitors. Given that Q_o site inhibitors can influence potential of heme b_L, this result implicates that electron equilibration within hemes *b* and the occupant of the Q_i site can effectively modulate amounts and properties of SQ_i. This is discused in the context of the mechanism of catalytic reactions at the Q_i site.

Levels of alpha amylase inhibitors and toxic fractions of gluten in sourdough bread

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Improper diet habits and physical inactivity are the main reasons of diet-related diseases. To avoid the risk of these conditions scientists recommend reduction in consumption of products rich in saturated or trans fats, refined grains, high in sugar and salt, as well as, an increase in intake of fresh fruit, vegetables and whole grains. Consumption of fermented foods is also recommended. Celiac disease and diabetes affect great part of world's population which increases demand for novel treatments and ways to improve patients' welfare.

Presented study focused firstly at six types of wheat sourdough bread produced by Bakery X in comparison to wheat bread prepared without leavening. All experiments were carried out to examine potential pro-health properties. In the second part, six different types of wheat sourdough Ciabatta rolls from Bakery X were analyzed after being subjected to *in vitro* digestion.

Obtained results showed increased levels of 33-mer (toxic fraction of gluten that is harmful to celiac patients) in analyzed bread samples, which can be a result of lactic acid fermentation. Although this may not look good for patients it may suggest that sourdough bread may be more susceptible to enzymes in the digestive system. This hypothesis was confirmed during immunoreactivity assay with previously digested Ciabatta rolls. Acidification and proteolysis of flour decreases the level toxic epitope twice.

Study also showed that the sourdough bread contains more α-amylase inhibitors in comparison to the control (Tostowy bread). All analyzed samples showed inhibition between 7 and 20%. Samples analyzed after *in vitro* digestion confirmed that baked goods containing sourdough show higher inhibition levels.

Upon presented experiment, examined type of wheat bread can be recommended to the patients in high-risk group and families with celiac disease history, as well as, to the limited extent to diabetics.

Cheese whey permeate as a renewable substrate for polyhydroxyalkanoates synthesis

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With the exhaustion of fossil fuels and increasing environmental issues, an alternative to conventional petrochemical plastics has attracted much attention in recent years. Especially, there is a growing interest in the group of biopolymers known as polyhydroxyalkanoates (PHAs).

They are accumulated by microorganisms in the form of granules under unbalanced growth conditions. Despite the highly satisfactory properties of PHAs, their production on a large scale is currently limited mainly due to high production costs. Inexpensive carbon sources such as wastes from dairy industry are taken into consideration to make PHAs biosynthesis more economical.

Therefore, the aim of this study was to investigate the ability of *Paracoccus homiensis* to synthesize polyhydroxyalkanoates during cultivation on cheese whey mother liquor.

The results showed that *Paracoccus homiensis* was able to synthesize biopolymers utilizing cheese whey mother liquor. It has been confirmed that the obtained biomass value and PHAs cellular content were dependent on the carbon source concentration. The data demonstrated that the analyzed bacteria was capable of accumulating PHAs up to 13.3 % of cell dry weight during the cultivation supplemented with 90 mL/L of cheese whey mother liquor.

In conclusion, our results clearly demonstrate that *Paracoccus homiensis* is a suitable organism for additional PHAs research because of its ability to grow and to accumulate polyhydroxyalkanoates on waste carbon sources like cheese whey mother liquor.

Preliminary electrochemical study of azathioprine interaction with DNA

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Azathioprine (AZA) is an immunosuppressive prodrug (a precursor of 6-mercaptopurine) from thiopurines group, commonly used in the treatment of autoimmune diseases or to prevent the rejection of organ transplantation. Presented results focuses on the electrochemical activity of azathioprine and its possible interaction with DNA. This hypothesis was verified with the use of bare working electrodes fabricated by direct printing method. Firstly, the studies of electrochemical behavior of AZA by means of cyclic voltammetry showed that it undergoes irreversible one-step electrochemical reduction. Then, the bare working electrodes were tested for quantitative determination of AZA with the use of differential pulse voltammetry. Due to the fact that azathioprine was successfully detected at the micromolar level with both, graphite and gold paste amperometric sensors, an attempt was made to choose the adequate (bio)receptor to obtain a selective response. As the first biorecptor DNA oligonucleotides were choosen. Therefore for further investigations, electrochemically active graphite surface was biofunctionalised with DNA-type bioreceptor. Effectiveness of the modification procedure was confirmed with the use of FTIR spectra analysis. In addition, the developed DNA based biosensors were examined by means of voltammetric measurements with the use of methylene blue as an intercalator. Electrochemical investigation of azathioprine-DNA interaction revealed slight negative potential shift indicating on electrostatic forces participation in complex formation, most plausibly through groove binding. The presented results suggest that DNA biofunctionalization of amperometric sensor surface enables the preparation of a selective analytical tool for determination of AZA in human body fluids.

The effect of newly elaborated synbiotic preparations on the genotoxicity of chicken faecal water

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The aim of the research was to evaluate of effect of three new elaborated synbiotic preparations (A, B and C) on genotoxicity of chicken faecal water on the cell line LMH (*Leghorn Male Hepatoma*).

Synbiotic preparations contained *Lactobacillus* sp. bacteria, *Saccharomyces cerevisiae* yeast and inulin. Experimental work was performed on 504 broiler chickens. Animal studies were conducted in Department of Pathology and Veterinary Diagnostics, SGGW, Poland. The faecal water (FW) of experimental animals (with administration of synbiotics) and comparative control animals (without intervention) and with the administration of commercial probiotic preparations (BioPlus® YC and Cylactin®) were analyzed. Using the comet assay (SCGE - *single cel gel electrophoresis*) the extent of DNA damage in the faecal water of chickens was determined. FW was extracted from fresh faecal samples of chickens (7 individuals from each group), collected on the 7 th and 42 nd day of life. An analysis of 100 randomly selected comets in each sample was done. The parameter tested was the percentage of DNA in the tail of the comet.

The genotoxicity of chicken faecal water due to feed additives in diet of animals was varied. The average genotoxicity of FW in chickens fed of fodder with synbiotics was from 16.20 ± 1,09% to 22.81 ± 1,24% and was lower than in the case of probiotic preparations (20.44 ± 1.36% - 22.86 ± 1,66%) and the control group (20.24 ± 1,27%). Synbiotic B and C are more effective in reducing the genotoxicity of chicken faecal water than commercial probiotics tested and the most effective was synbiotic B.

New elaborated synbiotic preparations have beneficial effect on decrease of extent of DNA damage in the chicken faecal water after 42 days of feed suplementation. Furthermore, the extent of genotoxicity reduction of faecal water in chickens is a highly individual feature.

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α-mangostin, a natural compound from *Garcinia mangostana* Linn exerts selective toxic activity against squamous carcinoma cells through inhibition of proliferation, adhesion and apoptosis induction

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α-mangostin is one of the major xanthones isolated from pericarp of mangosteen (*Garcinia mangostana* Linn), a tropical plant found in South East Asia that has been used as traditional medicine. Numerous *in vitro* and *in vivo* studies have revealed that α-mangostin exhibits a wide range of pharmacologic activities, including antioxidant, anti-inflammatory, antimicrobial as well as anticancer.

In this research, α-mangostin was investigated for an antitumor activity against squamous carcinoma (SCC-15) cells in comparison to normal fibroblasts (BJ). Parameters such as cytotoxicity, proliferation, adhesion, apoptosis and ATP level were studied after treatment of the cells with α-mangostin in the range of concentrations 2.5 – 40 μM.

α-mangostin revealed concentration- and cell type-dependent cytotoxic effect, much higher against cancer than normal cells. IC_{50} for SCC-15 cells was equal 6.43 μM (NR assay) and 7.72 μM (XTT assay). The toxic effect of α-mangostin in SCC-15 cells caused the disruption of the cell membrane integrity and mitochondria functions. Anticancer activity of α-mangostin was also manifested by inhibition of cells proliferation from 7.5 μM concentration after 72 h treatment. α-mangostin also reduced cells adhesiveness to 45% at 10 μM. Additionally, α-mangostin induced apoptosis both in cancer and normal cells, but in squamous carcinoma cells this process has begun earlier and was accompanied by ATP decrease.

Performed studies displayed strong selective cytotoxic activity of α-mangostin against squamous carcinoma cells by acting on different cellular parameters. For this reason, it is worth to pursue further research with α-mangostin and consider usage as an anticancer agent in an alternative targeted treatment of human skin cancer.

Role of Hrr25 kinase in the regulation of the Elongator complex

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Kinases regulate a variety of cell responses to external and internal stimuli via protein phosphorylation. Hrr25 is a yeast serine-threonine kinase that belongs to casein kinase I family. This protein is indispensable for many cellular processes, including vesicle trafficking, autophagy and chromosome separation. In addition, recently Hrr25 was observed to regulate tRNA modification process.

tRNA molecules are heavily modified. One of these modifications, 5-carboxymethyl (cm^5) group, is placed by a highly conserved Elongator protein complex to the U_{34}, which is located at the wobble position in the anticodon of specific tRNA species. The cm^5U_{34} modification is essential for an appropriate translation rate and protein folding. To perform its function, Elongator requires multiple accessory proteins, namely Kti11/13, Kti12 and Hrr25. Although Hrr25 is thought to be a potential switch between an active and inactive state of the Elongator complex, the precise role of this kinase is still elusive.

Here, we map the interaction between Elongator and Hrr25. We show that Hrr25 does not directly interact with Kti12. We prove that the purified kinase is capable of autophosphorylation. We utilize a PhosTag assay to track autophosphorylation and phosphorylation of individual domains of Elongator protein 1 by Hrr25. Furthermore, we analyse phosphorylated proteins using mass spectrometry to retrieve sequence-specific information about phosphorylation sites. Additionally, our biochemical studies are supported by *in vivo* phenotypic assays in yeast.

Our results confirm Hrr25/Elp1 interaction and that it is independent on other regulatory subunits. Available structural information together with detected phosphorylation sites allowed us to create a potential model of action of Hrr25 on the Elongator complex. First insights of *in vivo* studies have shown, that some phosphorylation sites play a critical role in kinase function, which may have multiple implications in intracellular signalling.

The impact of nZVI on *Lolium westerwoldicum*

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The increasing number of landfills, production plants, oil fields, mines and industrial plants causes more pollution enter the ecosystem. One of the biggest threats is the insertion of harmful compounds into the soil and further into aquifers. A relatively new solution is the use of nanoremediation. The last few years have brought a lot of reports about the high efficiency of nano zero-valent iron (nZVI) used for the purification of soil and water environments from heavy metals and other pollutants. Unfortunately, there is still a lack of data on the impact of these compounds on living organisms, including plants.

This study was designed to test the effects of nanoFER 25 iron on *Lolium westerwoldicum*. After cultivation of plants on a soil contaminated with nanoparticles, the biometric parameters, enzymatic and non-enzymatic antioxidant system were examinated. The conducted experiment showed that nZVI are slightly taken from the soil to the plants. Probably, iron accumulates in the outer layers of the roots or aggregates on their surface and closes further access to the tissues. At the same time, it can be observed that nZVI have no toxic effect on plant growth. The content of polyphenols and flavonoids in aboveground parts of plants decreases with simultaneous increase in roots compared to the control sample. It is possible that the plant transports these compounds to tissues that have most frequent contact with contaminants. The amount of vegetable dyes in the leaves increases for low extent of contamination and then decreases at higher levels of contaminants. Similarly, the enzyme activity of the antioxidant system in the whole plant is strongly related to the concentration of the pollutant.

The results are compatible with the literature but do not give a definite answer whether the use of nZVI is completely safe for ryegrass (*Lolium westerwoldicum*).

Multidirectional MTBE extraction

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Metabolomics is a developing area that allows profiling of samples from living organisms in order to gain insight into biological processes. The most important aspect of metabolomics is the sample preparation, a lot of controversial methods generate unreliable results.

Owing, not only to the complexity, but also to the diverse physicochemical properties of the cellular constituents, especially the different metabolite classes, no single extraction solvent can extract all molecular components from a complex biological sample. Accordingly, different classes of compounds require specific extraction methods to obtain adequate coverage of the full diversity of cellular metabolism.

To overcome large sample amounts the one-step extraction using tert-Butyl methyl ether (MTBE) was applied. The method was tested on wheat shoot tissues and microbial cell. The procedure allows for the lipids, primary and secondary metabolites analyzing. Chromatographic techniques compressed with tandem mass spectrometry were used for analysis (LC-MS/MS).

Methods based on chloroform extraction, leads to undesirable interphase between the polar and lipid phases, and produce amounts an undesirable solvent in terms of green chemistry. According to our results the solvent MTBE overcomes both the above problems and is a suitable substitute for chloroform.

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Molecular detection of *Anaplasma* spp. in game animals

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Anaplasma sp. is Gramm–negative bacteria, which is an obligatory intracellular parasite of animals and humans. The largest reservoir is made up by small rodents and wild animals e.g.: wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), and red deer (*Cervus elaphus*). The widespread occurrence of this potentially pathogenic bacteria in the environment can be a serious problem for human health. *Anaplasma* sp. is the etiological agent of anaplasmosis (GA – granulocytic anaplasmosis) – which is a zoonosis caused by direct contact with sick animal or indirectly by ticks (tick – borne disease).

The aim of this study is detection of *Anaplasma* spp. in the liver and spleen from randomly selected: wild boars, red deer and roe deer. 20 animals were randomly selected from each species, which livers and spleens were analyzed in parallel (60 animals, 2 organs, n = 120). Molecular analysis based on the variations of polymerase chain reaction (PCR, nested – PCR).

Preliminary studies showed the presence of *Anaplasma phagocytophilum* in the tested samples (wild boars). Several strains were identified as potentially pathogenic to humans. Through to the conducted research, it can be concluded that wild animals occurring in Poland are a reservoir of pathogenic bacteria from *Anaplasma* genus.

RNA purification and selection of stable reference gene in qPCR technique – why it matters?

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The qPCR technique is a highly sensitive, high-throughput and highly-reproducible method. In qPCR experiments, several types of controls should be applied along with the studied samples, these include: no template control (NTC) and RNA samples. The last one helps to exclude DNA residues contamination that might remain after DNase treatment during the RNA isolation process. Unfortunately, it is often overlooked which can seriously affect the produced results in quantitative gene expression analysis. The next major problem in qPCR method is selection for the best and stable reference gene. Many researchers base their choice of a reference gene exclusively on the already published data. Is it a good approach? Is there a universal gene, stable in every experimental condition?

The presented research focused on *Staphylococcus aureus* strain producing *sea* toxin. *S. aureus* cells were treated with visible light, and nontoxic chemical agent (a photosensitizer) (the so-called photodynamic inactivation - PDI). Two combinations of PDI experiments were used: rose bengal (RB) activated with green light (λ_{max}=515 nm) and new methylene blue (NMB) activated with red light (λ_{max}=632 nm). In PDI experiment, *S. aureus* strain was treated under the sub-lethal conditions, which do not significantly affect the survival of bacterial cells. Then, the total bacterial RNA was isolated. This step was carried out with two combinations: with and without additional DNase treatment. To obtaining the complementary DNA (cDNA), reverse transcription was conducted. In the qPCR technique, five reference genes: *16S rRNA*, *gmk*, *pyk*, *fabD*, *tpiA* were tested. For all of the primers pairs of reference genes, standard curves were performed with a serial dilution of cDNA. Genes stability were evaluated using three software programs: BestKeeper, geNorm and NormFinder.

The proposed experiment allowed answering the following questions: Is the 16S rRNA gene a universal gene? And how RNA purification affects Cp values?

Non-antibiotic therapy for neonatal Group B Streptococcus prophylaxis

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About 30% of women are colonized with *Streptococcus agalactiae* known also as a Group B Streptococcus (GBS). During the labour it can be transmitted to the newborn and cause sepsis, pneumonia or meningitis, what makes it one of the main causes of mortality in neonates. Because of the progression of antibiotic resistance there is a huge demand for alternative therapies. Our aim is to assess the potential of antimicrobial photodynamic inactivation (aPDI) in eradication of *S. agalactiae* in the urogenital tract. aPDI is based on the use of non-toxic photosensitizer which can be excited by the visible light. That causes generation of reactive oxygen species what finally results in bacterial cell death. We observed that aPDI with Rose Bengal (RB) as a photosensitizer can successfully eradicate *S. agalactiae in vitro*. We also observed no significant effect of this method on viability of the human keratinocytes. As a following step we performed 10 subsequent treatments of *S. agalactiae* population with a sub-lethal doses of aPDI with RB. The bacterial population from different time points is characterized with increased tolerance for aPDI. It has also altered response for treatment with hydrogen peroxide. This study reveals possibility of occurrence of bacterial tolerance for aPDI treatment.

Immunocytochemical analysis of the specificity of EGFRvIII binding L8A4 antibody in tumor cell lines

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EGFRvIII is an oncogenic version of EGFRwt. Mutated receptor occurs only in tumor cells. EGFRvIII is a good candidate for targeted therapy, therefore it is important to find a tool that allows its specific detection. Despite the commercial availability of several antibodies directed against EGFRvIII, none of them is fully specific for this antigen. The aim of this study was to check specificity of mouse monoclonal antibody L8A4 against EGFRvIII in tumor cell lines.

The analyses were performed on two glioblastoma cell sublines (DK-MG with high and low concentration of EGFRvIII protein) and on non-small-cell lung cancer cell line (H1975) as well as breast cancer cell line (MDA-MB-468) wherein the last two lines do not show endogenous expression of EGFRvIII. Real-time qRT-PCR analysis was performed in order to assess level of EGFRvIII and EGFRwt mRNA in DK-MG high and low sublines. Immunocytochemical (ICC) and Western Blot (WB) analysis were performed to determine specificity of L8A4 to bind only with EGFRvIII protein but not with EGFRwt protein.

ICC analysis on DK-MG sublines showed that L8A4 antibody detects probably only EGFRvIII variant. The signal in DK-MG high subline was much stronger than signal in DK-MG low subline, which is consistent with the results of EGFRvIII and EGFRwt mRNA expression from the Real-time PCR analysis. In H1975 cells, the nonspecificity of L8A4 binding was noticed. In the case of MDA-MB-468 during ICC only minimal fluorescence was observed. The results of Western Blot indicate that L8A4 may bind nonspecifically to EGFRwt.

It is uncertain whether L8A4 shows fully specificity for EGFRvIII. Our ICC analysis indicates that L8A4 binds preferentially to EGFRvIII but not to EGFRwt. However, WB results undermine the specificity of L8A4. The improvement of the antibody specificity may contribute to the development of effective targeted therapy against EGFRvIII.

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When bacteria circulate – microfluidic application for screening of bacteria cultures

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Microfluidic techniques are a powerful tool and are currently used for many applications. Numerous advantages that they offer are the reason why various disciplines of science reach for them more and more often. High throughput, possibility to make many parallel assays and automation, low reagents consumption and cost reduction are some features of them. The undeniable benefit of microfluidic systems is also the ability to adapt them to the needs of the experiment. Herein we present a self-prepared two-phase microfluidic device for continuous recirculation of droplets in a closed loop. We achieved a stable temperature and constant condition of gas exchange in the automated capillary-based fluidic system with the possibility of real-time optical characterization of droplet size and bacterial growth in a single droplet. As an example of application, we have made an experiment during which we measured the optical density (OD) of the thirty droplets with bacteria inside over the period of 336 h and prepared a growth curve. We compared it with the growth of bacteria breeding in droplets that flowed back and forth and we received a greater maximum growth rate in the current experiment. To check the potential of the device, we also checked the influence of different chloramphenicol concentrations on the growth of bacterial culture. The growth curves were dependent on the antibiotic concentration. After the research, no signs of contamination were found. This makes the device suitable for various applications in the future.

Evaluation of the phage-antibiotic synergy (PAS) based on different variables of the double-layer agar (DLA) method

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With the increase of the antibiotic resistance frequency and the decreasing frequency of new antibiotics being developed, alternative strategies to antibiotic therapy are urgently needed by the worldwide medical and scientific community. Bacteriophages, which are considered to be the natural enemies of bacteria, have recently gained the interest as therapeutic agents. Phage therapy is based on the use of lytic phages to combat bacterial infections, including multidrug-resistant bacteria and has many advantages, as phages persist, as long as the targeted bacteria are present, they are also very specific and efficient for their bacterial host. That reduces destruction of the natural flora and presents no detrimental effects on human cells. Therefore, some researchers became interested in using phages in combination with current antibiotics and have demonstrated that this approach, can be a promising way to improve antimicrobial activity. This phenomenon is known as phage-antibiotic synergy (PAS). Although the lytic activity of phages has been shown to be synergistically enhanced in the presence of antibiotics, PAS is still not clearly understood. The research carried out so far was different concerning the method of modifying the double-layer agar (DLA) assay to show the PAS effect.

Therefore, in this work we assessed the modification of DLA method, which would be the most effective to visualize the PAS effect. This study shows that the choice of the antibiotic addition placement (to the bottom or top agar) affects the PAS recognition, and the presence of the bottom agar have an influence on the PAS effect evaluation.

Double-head cationic lipopeptides as the effective antimicrobial agents

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The discovery of antimicrobial agents was a milestone and changed the face of medicine forever. Accidental invention of penicillin allowed effective treatment of diseases caused by bacteria, which were considered to be deadly or leading to irreversible damage to the body. Patients suffering from commonly occurring diseases such as tuberculosis, syphilis or gonorrhea could be effectively treated with newly discovered antibiotics. The searching for new, effective compounds with antimicrobial properties is a big challenge for modern science.

The promising class of compounds are antimicrobial peptides (AMPs), which are an important element of the immune system of all living organisms. A specific structural feature of most antibacterial peptides is the presence of strongly basic amino acid residues (Lys, Arg), and in the case of antifungal compounds, the presence of multiple histidine residues.

The previous studies have shown that the combination of short cationic peptides with fatty acid residues improves or even gives the compounds obtained antimicrobial properties. As a part of this study, symmetrical lipopeptides, containing palmitic acid attached to the center of the peptide using different lengths of linkers, were synthesized. The obtained compounds were tested for antimicrobial properties and their interaction with the model lipid membrane.

The synthesis of polyhydroxyalkanoates by *Paracoccus homiensis* from volatile fatty acids

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Polyhydroxyalkanoates (PHAs) are bacterial polymers accumulated intracellularly as a source of energy. Due to their biodegradability, thermoplasticity or biocompatibility, PHAs have potential in agricultural, industrial and biomedical applications. PHAs commercial production is currently limited because of the high production costs compared with synthetic polymers. There is still a growing need to improve PHAs productivity by bacteria using different carbon sources. Therefore, the aim of the present study was to investigate the ability of *Paracoccus homiensis* to synthesize polyhydroxyalkanoates during growth on volatile fatty acids (VFAs) such as acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid and caproic acid.

The results indicated that the volatile fatty acids could support cell growth of the *Paracoccus homiensis*. The highest concentration of cell dry weight (1.77 g/L) was observed during growth with caproic acid. Furthermore, biopolymers content in the bacterial cells was dependent on the type and concentration of VFAs used. The highest amount of PHAs (20.0 %) was obtained using valeric acid at a concentration of 4 g/L.

The conducted research allowed to estimate the effect of various concentrations of volatile fatty acids on the growth of *Paracoccus homiensis* and the effectiveness of PHAs synthesis and accumulation. As a result of the screening carried out, the most intense accumulation of biopolymers was noted using caproic acid.

Immobilization of recombinant hydrolytic domain of PelA from *Pseudomonas aeruginosa* involved in biofilm formation on bacterial biopolymer as a carrier

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Most microorganisms living in natural environment create their own microenvironment called “biofilm”. In biofilms microorganisms produced extracellular substances called “matrix” which contain polysaccharides as a major component. Biofilm matrix has many variety functions i.e initial surface adhesion, aggregation of bacterial cells mechanical stability, and protection against antimicrobial agents and environmental stressors which causes that bacterial infections are very difficult to cure. Thus, degradation of the biofilm matrix is necessary to effective action of antibiotic and killing the bacteria. One of the most promising biofilm eradication biological method is using an enzyme especially glycoside hydrolase which act by hydrolyzing the glycosidic linkages between two or more carbohydrates. The composition of the biofilm matrix differs significantly between bacterial species. The best-known example is *Pseudomonas aeruginosa*, which contains at least three different polysaccharides in biofilm matrix Pel, Psl and alginate. Structure of Pel polysaccharide has not been fully known yet. Carried out analysis suggest that Pel is a glucose – rich polysaccharide and its synthesis machinery is encoded by a seven gene in operone (*pelA-F*). PelA, the first gene in the operon, is a periplasmic protein involved in modifying Pel polysaccharide and it is a multi-domain protein with at least four catalytic activities. One of the domain is glycoside hydrolase which can be used to cleave Pel polysaccharide and disrupt *Pseudomonas aeruginosa* biofilms.

The aim of the present work was expression of *pelA* gene for $PelA_h$ purification, an enzyme involved in *Pseudomonas aeruginosa* biofilm disruption and its immobilization on microbial biopolymer based carrier. The obtained system was further analyzed for characterization of PelAh antibiofilm activity.

Examination of the dynamics of the *Leishmania major* pteridine reductase 1 enzyme in complex with methotrexate

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Leishmania is a Trypanosomatid parasite that is a significant public health problem due to widespread zoonosis. Current treatments are inefficient mainly because of drug resistance and side effects. Therefore, there is a need to find new ways to tackle Leishmania. Inhibition of the trypanosomatid folate pathway enzymes is a promising strategy and the drug methotrexate (MTX) is considered as a potentially interesting compound against leishmaniasis. One of the mechanisms of action of MTX is based on inhibition of the folate pathway enzyme dihydrofolate reductase (DHFR). However, trypanosomatids have another enzyme - pteridine reductase 1 (PTR1) - which acts as a metabolic 'bypass': the PTR1 enzyme confers resistance to drugs that inhibit DHFR. Consequently, there is a need to explore how antifolate compounds interact with PTR1. Thus, we here study MTX as an inhibitor of Leishmania major PTR1 by carrying out molecular dynamics simulations to investigate the effects of MTX binding on the structure and dynamics of the Leishmania major PTR1 homotetrameric enzyme.

Bacteria as microbuilders - sand bioconsolidation processes

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The term biomineralization refers to the processes in which living organisms produce mineral structures. One of the most important biominerals connected with this process is calcium carbonate ($CaCO_3$). The most simple mechanism during which calcium carbonate is precipitated is urea hydrolysis catalyzed by the enzyme – urease (urea aminohydrolase, E.C. 3.5.1.5). This mechanism is associated with the process of bioconsolidation which thanks to the introduction of microorganisms -microbuilders, that produce soil particles binding materialsis soil strengthening.

The aim of research was to determine the influence of granularity of four types of sand on the consolidation process induced by bacteria: *Bacillus muralis*, *Oceanobacillus massiliensis*, *Paeniglutamicibacter sulfureus*, and *Psychrobacillus psychrodurans*. In the first stage of the research, the urease activity of the tested bacterial strains was measured using a colorimetric method. The highest urease activity was found in the *Paeniglutamicibacter sulfureus* strain whereas the lowest in the *Oceanobacillus massiliensis*. Moreover, in this study the effect of both granulation of sand in the range of 0.1 - 1.0 mm and the type of calcium source in cultivation medium on the process of bioconsolidation was examined.

The process of bioconsolidation depends on bacterial strain, the source of calcium ions, the presence of urea and the degree of graininess of the soil. The advantages of bioconsolidation, such as low costs or no adverse environmental impact, make it an innovative method that is becoming more popular and is used in many fields, including civil engineering and geotechnology.

Analysis of interactions between nucleotide sugar transporters using the NanoBiT system

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NanoBiT is a novel technique that can be used to measure protein-protein interactions in living cells. The NanoBiT system is composed of two small subunits (one of them is only 11aa-long) that are expressed as fusions to the proteins of interest. When the target proteins are in close proximity, the subunits form an active luciferase enzyme which generates a luminescent signal in the presence of substrate. For the first time we used this system to study interactions of the Golgi apparatus multitransmembrane proteins involved in the transport of nucleotide sugars in mammalian cells. We confirmed that the UDP-galactose transporter (UGT) forms homodimers and/or higher oligomers. Additionally, we showed that UGT variants tagged with the NanoBiT fragments localize properly and remain functional. Finally, we demonstrated interactions between the UGT and the UDP-*N*-acetylglucosamine transporter. In conclusion, we suggest that the NanoBiT system is a useful tool with a great potential to study interactions between Golgi membrane proteins in living cells.

The molecular characterization of *Listeria monocytogenes* strains isolated from fish and fish processing plants

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Listeria monocytogenes are Gram-positive bacilli, commonly found in the natural environment. These bacteria cause listeriosis. Food is the main source of *L. monocytogenes*. The molecular biology methods are widely used in clinical and industrial microbiology, e.g. for identification and characterization of microorganisms.

The aim of the study was the molecular characterization of *L. monocytogenes* strains isolated from fish and fish processing plants.

The research material consisted of 37 *L. monocytogenes* strains isolated from fish and fish processing plants and the reference strain *L. monocytogenes* IW 41. Species identification, the frequency of selected virulence genes and serogroups classification was determined using the multiplex-PCR technique. The genetic (PFGE, RAPD method) and protein similarities (MALDI-TOF MS technique) of isolates were determined.

The presence of *rrs* and *hlyA* genes was demonstrated in all examined isolates, which confirmed that they belonged to the *L. monocytogenes* species. Among 37 *L. monocytogenes* isolates, 36 strains were found, one of which included two genetically identical isolates (PFGE, RAPD method). In all examined strains (n=36, 100.0%), the following genes were found: *hlyA*, *plcB*, *plcA*, *inlA*, *inlB*, *prfA*, *iap* and *actA*. The presence of virulence genes, *mpl*, and *fbpA* was confirmed in 32 (88.9%) strains. It was reported that 30 (83.3%) of the strains belonged to serogroup 1/2a-3a.

Assessment of the virulence level of *L. monocytogenes* strains is an important aspect of public health protection, especially since there has been an increase in the number of patients with listeriosis in recent years. In our study, it was found that strains with a high degree of genetic similarity also showed a high degree of similarity at the level of protein profiles. The molecular biology methods allow quick detection and characterization of pathogens, and these are an invaluable tool in epidemiological studies.

Determination of anti-adhesive and antimicrobial properties of *Vaccinium macrocarpon* berry extract towards *H. pylori* ATCC 43504 strain

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Introduction. The number of antibiotic-resistant *Helicobacter pylori* (*H. pylori*) - bacteria that cause stomach/duodenal ulcers is growing constantly, inspiring the scientists to search for an alternative methods to prevent such infections.

Aim. Determination of the antimicrobial and anti-adhesive activity of *V. macrocarpon* berry extract towards *H. pylori* adhesion to gastric epithelial AGS cells.

Materials and methods. The antimicrobial activity was evaluated according to broth microdilution method (ISO 20776-1-2006) towards *H. pylori* ATCC 43504 reference strain. To determine anti-adhesive potential AGS cells ($2x10^5$ cells/ml; 100 µl/well) were incubated overnight, washed twice and exposed to cranberry extract (256 µg/ml 1.28 µg/ml), for 30 min. Then the cells were washed twice and fluorescently labeled *H. pylori* suspension was added to selected wells (100 µl/well) and incubated for another 30 min. The unbound bacteria were washed out and the intensity of fluorescence was measured (λEm/Ex=516/480 nm). Cells were stained and visualized in confocal microscope.

Results and conclusion. The MIC of *V. macrocarpon* extract was 6.25 µg/ml. It was also shown that *V. macrocarpon* berry extract used in the MIC concentration and other doses reduces the adhesion of *H. pylori* bacteria to gastric epithelial cells. This phenomenon was observed in the range of concentration–1.28 µg/ml–3.84 µg/ml and 15.36 µg/ml–256 µg/ml. We have observed a 58.1% decrease in fluorescence in cultures pretreated with cranberry extract (1.28 µg/ml), in comparison to the AGS cells incubated with bacteria without this component: 1792733±179969.7 vs 1060292±12344.2. The cranberry extract block the *H. pylori* adhesion to gastric epithelium, which makes it a potential candidate for *H. pylori* prevention agent.

This study was conducted as a part of „Microscopic Imaging Techniques”; laboratory classes and was co-financed by the European Regional Development Fund under the Smart Growth Operational Programme 2014-2020.

Synthesis of peptide stabilised gold nanoparticles

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Nanoparticles are emerging as a promising class of therapeutics for cancer. Clinical results suggest that therapeutics based on nanoparticles can show enhanced efficacy, while simultaneously reducing side effects arising from its properties, such as more targeted localization in tumours.

The aim of the project is to obtain peptides-capped nanoparticles, which act as an intracellular delivery of anticancer agents to tumor tissues. There are many publications, which indicate glutathione-stabilized gold nanoparticles, making them properly examined. Glutathione-stabilized gold nanoparticles synthesis distinguish from its stability. Moreover glutathione occurs in human body. It is needed to transform reactive oxygen species into harmless form, what stands out for good assimilability of glutathione-stabilized gold nanoparticles. Furthermore, glutathione has an amino group (-NH2), a thiol group (-SH) and carboxyl groups (-COOH), which set it as a perfect component to assemble with the therapeutic entities.The synthesis features use of yeast extract instead of pure glutathione. Yeast extract contains from 5 up to 7% of glutathione, what gives the opportunity to optimize synthesis and make it more economical and eco-friendly. Usage of gold nanoparticles also provides entire removability from the organism[1].

Results show that changing parameters of the synthesis, such as reducing quantity of yeast extract or using buffer solutions influence on physical and chemical properties of gold nanoparticles. Received nanoparticles have been characterized by SAXS, TEM, and UV-Vis analysis.

[1] C.A.Simpson, In vivo toxicity, biodistribution, and clearance of glutathione-coated gold nanoparticles, Nanomedicine, 257–263(2013).

Dual fluorescent indicator for determination of B-glucosidase activity

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The approaches enabling determination of low-activity enzymes are of high interest to biochemists and biophysicists. Design of selective enzyme indicators and techniques of their sensitive detection require combination of new approaches, especially when extremely small quantities or low activity enzymes are available.

Fluorescence spectroscopy is one of the most sensitive methods which enables "single-molecule" detection under specifically defined conditions. One of the main requirements for the fluorescent indicators is the change of fluorescence parameters under the action of the enzyme. Such an "ON/OFF" ratio of fluorescence intensity in the presence and absence of an enzyme defines sensitivity of the indicator. One of the best examples of "ON/OFF" signal separation can be assessed using the fluorophores which undergo Excited-State Intramolecular Proton-Transfer (ESIPT).

The presented results are continuation of our research on the determination of β-glucosidase activity using fluorescent probes based on 3-hydroxy-chromen-4-one derivatives, whose mechanism of action is sensitive to pH value. In this presentation I have proposed a new indicator, which combines the features of ESIPT and ordinary fluorophore. The research allow to specify two different mechanisms for designation the fluorescence intensity with pH change. In acidic environment, the enzyme activity can be monitored by two fluorescent signals derived from two neutral tautomeric forms, which coexist because of ESIPT phenomenon. In neutral environment, the main analytical signal is derived from the excited anionic form, which is created because of protolytic dissociation of the released fluorophore.

In summary, the presented probe allows a more accurate estimation of the activity of hydrolytic enzymes using two different detection methods by means of a change in pH.

Multicomponent system magnetite/lignin/polydopamine-glucose oxidase as functional biosensor for glucose detection

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The observed dynamic development of chemical technology and consumer demand implies the design and acquisition of more and more effective materials. An example of measuring devices that are increasingly used due to their high sensitivity and selectivity are biosensors. Biosensor is type of chemical sensor, which is using a biological recognition element. They are used to detect, transmit and record information that is processed into an analytical signal. There are several techniques used to immobilize the enzymes which allows for represent an expansion into many new fields of science and industry. The use of enzymes can be a cheaper and more available substitute for testing body fluids or environmental samples.

The aim of this work was the synthesis and optimization of the multicomponent system based on magnetite/lignin/polydopamine-glucose oxidase. The components used in the work are characterized by interesting properties that, combined with each other, create the effect of integrated operation. The obtained system is characterized by sensitivity, thermal stability, selectivity and furthermore is made of cheap, natural or of natural origin materials. The Fe_3O_4/Lig/PDA-GOx platform has been shown to have very good properties for glucose detection, due to good sensitivity and selectivity for other sugars. The creation of a system with the desired characteristics enables the detection of various substances, depending on the biological layer. The effectiveness of receiving the magnetite/lignin/polydopamine - glucose oxidase system has been confirmed by a number of physicochemical tests. Electrochemical measurements carried out as part of this work have proved that immobilized on the surface enzyme is catalytically active, moreover, analysis carried out on real solutions were characterized by repeatability of results.

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Cellulase activity in Superworm *Zophobas morio* (Coleoptera: *Tenebrionidae*)

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Current World energy needs demand the development of large scale production of fuel from renewable resources. Cellulosic ethanol is suggested as a desirable biofuel, due to its abundance and sustainability. However efficent production of bioethanol using funagal and bacterial enzymes is limited. There is growing evidence indicating that the insects cellulases can be utilised in this area. They produce endogenous and exogenous cellulases which hydrolyse plant biomass into the substrates used in biofuel production. The aim of this study was to determinate the biochemical characteristics of celulases extracted from Superworm *Zophobas morio*. Endo-β-1, 4-glucanase activity was measured using carboxymethyl cellulose (CMC) and exo-β-1, 4-cellobiohydrolase activity was measured using microcrystalline cellulose (MCC) as substrates. The results of this study indicated that optimum of cellulases activity is 50 °C and 2% substrate concentration in both larvae and imago. In conclusion this study found that cellulolitic complex from *Zophobas morio* have ability to hydrolyse cellulose suggesting its potential utilisation in biofuel production.

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The Symposium „Symbioza" takes place at the Warsaw University of Technology, at the Faculty of Mathematics and Information Science

Iskra club is located at Wawelska 5