PROGRAMME & ABSTRACT BOOK

FEBS3+ MEETING

„50 years of Recombinant DNA – Past, Present, Future“

XXVIITH BIOCHEMISTRY CONGRESS

OF SLOVAK AND CZECH SOCIETIES FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY WITH COOPERATION OF HUNGARIAN AND UKRAINIAN BIOCHEMICAL SOCIETIES

10. 9.-13. 9. 2023
High Tatras, Slovakia

SLOVAKIA FEBS3+ meeting

ORGANIZERS

SPONSORS

PARTNER

„50 years of Recombinant
DNA – Past, Present, Future“

XXVIITH BIOCHEMISTRY CONGRESS

OF SLOVAK AND CZECH
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FEBS3+ MEETING

PROGRAMME & ABSTRACT BOOK

SEPTEMBER 10th – SEPTEMBER 13th, 2023
High Tatras, Slovakia

CONTENTS

WELCOME

Dear participants,

it is with real pleasure that we proudly host the XXII th Biochemistry Congress - FEBS3+ meeting, which wil take place on September 10th--13th, 2023 in the amazing environment of the High Tatras in Slovakia. The congress bears the motto: „50 years of recombinant DNA - Past, Present, Future“ to commemorate the anniversary of the creation of the First Recombinant DNA. This newly born technology has become one the basic approach in various research areas such as healthcare, agriculture, industry, energy. The advent of recombinant DNA technology revolutionized the development and research of life sciences and led to a series of dramatic changes. A lot has happened since its discovery and it offered us new opportunities for innovation and was able to prepare many new molecules with the therapeutic effect of DNA.

The congress as a FEBS3+ meeting is organized by the Slovak Society for Biochemistry and Molecular Biology in close cooperation with the Czech Society for Biochemistry and Molecular Biology and with the organizational participation of the Ukrainian Biochemical Society and the Hungarian Biochemical Society. Excellent scientists from Europe are invited, the scientific sections of the congress will be held in English and their thematic structure reflects the latest trends in Biochemistry and Molecular Biology research.

Congress participants will be welcomed at the Grand Hotel Bellevue in the heart of the High Tatras in Horny Smokovec. Nature of High Tatras offers unusually clean air, mountain climate, wonderful fauna and flora. You will find glacial ponds, many beautiful hiking trails, many beautiful waterfalls, or the Belianska cave, the only accessible cave in the High Tatras. Thanks to this, the High Tatras received the prestigious award as the most important European destination of 2019, which they received from Lonely Planet.

We worked hard to make the event in the High Tatras an attractive congress for all participants. The program covers a wide range of current topics in the field of molecular life science, especially biochemistry, molecular biology and related sciences. To continue tradition of the biochemistry congress, the meeting wil feature basiic scientific sessions in the field of life sciences research including lectures of invited keynote speakers and oral communications selected from the submitted abstracts. In addition to an attractive scietific program we also promise to prepare an enjoyable social events.

We believe that this event will be a place for lively scientific debates, confirmation of existing research collaborations and establishment of new scientific contacts in the pleasant environment of beautiful Slovak nature. The goal is also to offer young scientists important information about FEBS, IUBMB, EMBL and can help them develop their future scientific careers.

We hope that your professional expectations will be fulfilled and you will attend many interesting lectures and meetings which will bring inspirations for your future research.

Ján Turňa
President of SSBMB

Gabriela Gavurníková
Stanislav Stuchlík
Zdenko Levarski
Local Organizing Committee

VENUE

The Grand GRAND HOTEL BELLEVUE Resort is located in the oldest settlement of the High Tatra Mountains – Starý Smokovec – at the altitude of 1,010 metres above the sea level. The site is known as Pekná Vyhliadka ("Beautiful View"), situated at the foothill of Slavkovský štít (Slavkovský Peak). The name of the hotel – BELLEVUE – is derived from the French translation of the site.

Present Grand Hotel Bellevue arose from two Tatra hotels – hotel Bellevue and Hotel Sport.

Grand Hotel Bellevue has been designed and built with the intention to offer exceptional opportunities. Due to the strategic location hotel can be easily accessed by car, train or plane.
From the European point of view the High Tatra Mountains are a unique combination of natural beauties, alpine environment with excellent conditions for tourism and skiing, as well as climatic spa combining healing effects of altitude with sunshine and fresh air.


XXVIIth Biochemistry congress
BELLEVUE
GRAND HOTEL

Featuring 150 guest rooms, congress hall for 750 participants, function rooms with flexible layout, wellness centre and sports facilities, Grand Hotel Bellevue is an excellent venue for your business, leisure and sports. Complete space fot 1200 participants.

The Multipurpose congress hall GRAND BELLEVUE, together with 5 smaller function rooms with flexible layout is an excellent place for organizing cultural, social or corporate events or family reunions. Total floor space of 1,000 square metres is suitable for international events, exhibitions and business presentations.

The congress hall can be divided into two separate conference rooms – BELLEVUE I. with the area of 395 square metres and seating capacity of 340 people and BELLEVUE II. with the area of 256 square metres and seats for 220 people.
It is our goal to bring you first class services that combine tradition with modern style:

- professional approach
- Slovak hospitality
- friendly staff


BELLEVUE
GRAND HOTEL · VYSOKÉ TATRY

COMMITTEES

CONGRESS CHAIR
Ján Turňa

CONGRESS CO – CHAIR
Libor Grubhoffer

CONGRESS ORGANIZING COMMITTEE
Gabriela Gavurníková
Stanislav Stuchlík
Zdenko Levarski
Alena Hajduchová
Anton Horváth
Miroslav Barančík
Irena Krumlová

SCIENTIFIC PROGRAMME COMMITTEE
Ján Turňa (Charmain)
Stanislav Stuchlík
Albert Breier
Erik Sedlák
Peter Račay
Zdenko Levarski
Gabriela Gavurníkova
Anton Horváth

SOCIAL PROGRAMME

Welcomme reception
in the Congres Venue

Sunday, 9th September 2023
Start: 19:00 (till 21:00)
Location: Congress Grand Hotell Bellevue
About the Event:
After the Opening ceremony we invite
all participants of the Congress
Menu : snacks and drinks
Price: included in the registration fee

Gala Dinner

Monday 11Th September 2023
Start: 19:30 (till 22:00)
Location: Congress Grand Hotel Bellevue
Menu: Hot dishes, snacks, desserts, hot
and cold beverages, wine, beer
Price 35 Eur
For the congress participants who
chose the Gala Dinner in the extra options

Social event - Wine tasting

Tuesday 12th September 2023
Start: 19:30 (till 22:00)
Location: Congress Grand Hotel Bellevue
Menu: Hot dishes, snacks, desserts, hot
and cold beverages, wine, beer
Price 45 Eur
For the congress participants who chose
the Social event

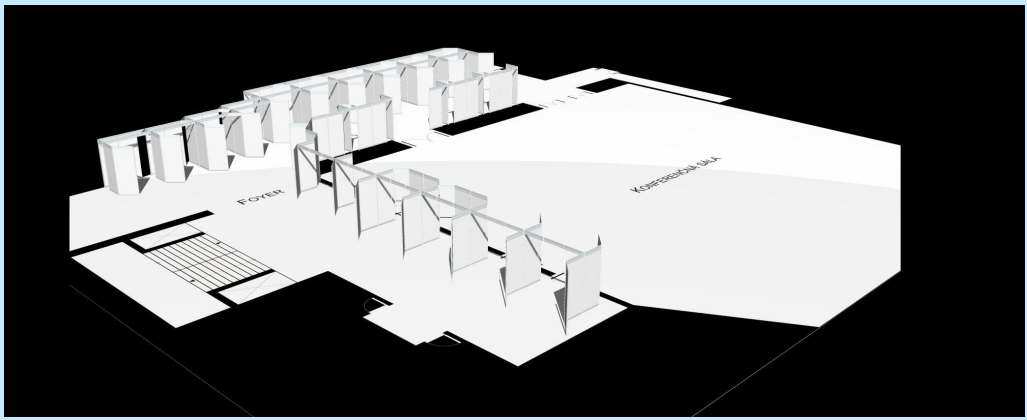
POSTERS SESSION

10th - 13th September 2023
Start : 18:00 - 19:00
Location : Foyer Grand hotel Bellevue
Posters discussion (The organizing committee will award three best posters at the FEBS3+ congress closing ceremony)

Poster size should not exceed 841 mm x 1189 mm (A0).
Posters should be mounted at the panels before 10:00AM
The poster boards will be available for the entire time of the duration of the congress and will be located in the foyer of the Bellevue hotel.

Young Investigators Poster Competition:
Posters of PhD. students in the Young Investigators Poster Competition (registered via the registration form **Student (ISIC card, ≤30 years)** will be presented during one of the poster sessions and evaluated by Poster Awards Committee. Presentors will present their results in front of their posters, presentations **should not exceed 3 minutes.**

Three best posters from the Young Investigators Poster Competition will be awarded with diploma and a financial award


Foyer
Konferenčna dvorana

CONGRESS PROGRAMME

Sunday 10.9.2023

10:00 - 18:30 **REGISTRATION** /Foyer Hotel Bellevue/Accomodation of participants - **14:00**

16:30 – 17:00 **OPENING CEREMONY (Congress Hall Grand Bellevue)**
Welcome (Ján Turňa – president of SSBMB, Libor Grubhoffer – president of ČSBMB)
Presentation of commemorative SSBMB medals
Award ceremony of Josef V. Koštíř for significant scientific contribution in the field of biochemistry and cellular and molecular biology
Ján Turňa (Department of Molecular Biolology, Comenius University, Bratislava): **50 years of recombinant DNA - past, present and future.**

17:00 - 18:00 **Plenary Lecture (Congress Hall Grand Bellevue)**
Andrea Fleig (Biomedical Research at The Queen's Medical Center Honolulu County, Hawaii, USA): **Cannabinoids as Modulators of Calcium Signaling and Inflammation.**

19:00 - 21:00 **Welcome Reception**

Monday 11.9.2023

8:00 -18.30 **REGISTRATION /Foyer Hotel Bellevue/**

9:00 - 9:50 **Plenary Lecture (Bellevue I)**:
Ulrike Stein (Experimental and Clinical Research Center, Charité - Universitätsmedizin Berlin and. Max-Delbrück Center for Molecular Medicine): **Restrict cancer metastasis - save patient life: Translating gene discovery into clinical application.**

10:00 -11:30 **Lectures in parallel Sections**

Molecular basis of disease and therapy **(Bellevue I)**
Martin Kolísek - chair
(Comenius University in Bratislava, Jessenius Faculty of Medicine)
Silvia Pastoreková – chair
(Biomedical Center SAS, Bratislava)

10:00 - 10:30 **Šárka Pospíšilová** (Medical faculty, Masaryk University, Brno)**: Molecular genetic changes in lymphoid malignancies and their impact on the pathogenesis, prognosis and molecular evolution of the disease (**ČSBMB J.V.Koštíř prize talk**).**

10:30 - 10:50 **Jörg Aschenbach** (Institute of Veterinary Biochemistry, Freie Universitaet Berlin, Germany)**: Role of magnesium in energy and calcium homeostasis of dairy cows - translations to humans**. - **invited speaker**

10:50 - 11:10 **Ralf Einspanier** (Institute of Veterinary Biochemistry, Freie Universitaet Berlin, Germany)**: Holistic analysis of gene expression (NGS, proteomics) and functions in animal diseases: Canine cancer and varroa treatment in honeybees as examples**. - **invited speaker**

11:10 - 11:30 **Jürgen Vormann** (Institute of Veterinary Biochemistry, Freie Universitaet Berlin, Germany)**: Magnesium and acid-base balance in aging. - invited speaker**

Cancer Cell Signaling, Regulations & Xenobiochemistry (Bellevue II)

Albert Breier - chair
(Centre of Biosciences SAS, Bratislava)
Miroslav Machala - chair
(Veterinary Research Institute, Brno)

10:00 - 10:30 **Miroslav Machala** (Veterinary Research Institute, Brno): **Novel aspects of Benzo[a]pyrenes toxicity.**

10:30 - 10:55 **Angéla Békési** (Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest): **Cell responses upon thymidylate synthase inhibitory treatments differ in multiple molecular and phenotypic aspects.**

10:55 – 11:20 **Jakub** Styk (Geneton Ltd., Bratislava, Slovakia, Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University): Unveiling The Potential Of Liquid Biopsy: **Non-Invasive Cancer Screening For Early Detection And Preventive, Predictive And Personalized Medicine.**

11:30 -12:00 **Coffee Break**

12.00 - 13.00 **Lectures in parallel Sections**

Molecular basis of disease and therapy (Bellevue I)

Martin Kolísek - chair
(Comenius University in Bratislava, Jessenius Faculty of Medicine)
Silvia Pastoreková – chair
(Biomedical Center SAS, Bratislava)

12:00 - 12:20 **Martin Kolísek** (Comenius University in Bratislava, Jessenius Faculty of Medicine): **Identification of multivariate mitochondrial fitness algorithm in diagnostics of Parkinson's disease.**

12:20 - 12:40 **Petra Hnilicová** (Comenius University in Bratislava, Jessenius Faculty of Medicine): **On the interface between muscle wasting and central neurodegeneration.**

12:40 - 13:00 **Zuzana Tatarková** (Comenius University in Bratislava, Jessenius Faculty of Medicine): **Aging and the role of MAMs in the protection of the postischemic heart**.

Cancer Cell Signaling, Regulations & Xenobiochemistry (Bellevue II)

Albert Breier - chair
(Centre of Biosciences SAS, Bratislava)
Miroslav Machala - chair
(Veterinary Research Institute, Brno)

12:00 - 12:20 **Ewelina Zarakowska** (Collegium Medicum Bydgoszcz, Nicolaus Copernicus University Toruń, Poland): **Impact of vitamin C supplementation on the epigenetic modifications in DNA and mRNA expression of vitamin C transporter genes in chronic lymphocytic leukemia.**

12:20 - 12:40 **Veronika Huntošová** (Faculty of Science, P. J Safarik University in Košice): **Autophagy and apoptosis switching during photo-activation of cancer cells.**

12:40 -13:00 **Albert Breier** (Faculty of Chemical and Food Technology Slovak University of Technology in Bratislava): **Interplay between P-glycoprotein mediated drug resistance and the LPHN1/GAL-9/TIM-3 signaling pathway**.

13.00 - 14.30 **Lunch**

14:30 -16:00 **Lectures in parallel Sections**

Recombinant protein production (Kristal Lounge)

Stanislav Stuchlík – chair

(Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava)

Michaela Wimmerová – chair

(National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno)

14:30 – 15:00 **Martin Toul** (Loschmidt Laboratories, Dept. of Exp. Biology, Faculty of Science, Masaryk University, Brno, 2. RECETOX, Faculty of Science, Masaryk University, 3. International Clinical Research Center, St. Anne's University Hospital Brno): **Rational protein engineering driven by kinetics**. **(**ČSBMB J.V.Koštíř prize talk**)**

15:00 – 15:20 **Ileana C. Farcasanu** (University of Bucharest, Faculty of Chemistry, Romania): **Hexatriacontahexapeptides designed as metal-binding Tags.**

15:20 – 15:40 **Veronika Hovanová** (Center for Interdisciplinary Biosciences, Technology and Innovation Park, P.J. Šafárik University, Košice, Department of Biophysics, Faculty of Science, P.J. Šafárik University, Košice, Department of Biomaterials, Faculty of Engineering Science, Universität Bayreuth, Department of Biochemisty, Faculty of Science, P.J. Šafárik University, Košice): **Modulating the properties of recombinant spider silk protein eADF4(C16) hydrogels.**

15:40 – 16:00 **Ľubica Kormanová** (Department of Molecular Biology., Faculty of Natural Sciences, Comenius University in Bratislava): ***Vibrio natriegens*****: A promising expression host for the production of "difficult-to-express" recombinant proteins.**

Postranslational Modifications, Glycobiochemistry **(Silver Lounge)**

Ján Tkáč - chair

(Institute of Chemistry SAS, Bratislava)

14:30 - 15:00 **Michaela Wimmerová** (National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno): **Protein glycosylation: synthesis, significance in health and disease, and its specific detection by lectins.**

15:00 - 15:30 **Filip Melicher (**Masaryk University, Brno**): Production and crystallization of lectin from *Photophabdus laumondii.***

15:30 - 16:00 **Ján Tkáč** (Institute of Chemistry SAS, Bratislava)**: Disease diagnostics by analysis of glycans.**

16:00 -16:30 **Coffee Break**

16:30 -17:30 **Lectures in parallel Sections**

Recombinant protein production (Kristal Lounge)

Stanislav Stuchlík - chair
(Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava)
Michaela Wimmerová - chair
(National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno)

16:30 - 16:50 **Eva Struhárňanská** (Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava): **Production of recombinant peroxidases, their characterization and potential use**.

16:50 - 17:10 **Marek Korsák** (Central European Institute of Technology, Masaryk University, National Centre for Biomolecular Research, Faculty of Science, Masaryk University): **Production of recombinant lectin PluLec from *Photorhabdus luminescens* for neutron crystallography and its structure-functional characterization.**

17:10 - 17:30 **Carlos Díaz** (Center for Interdisciplinary Biosciences – Technology and Innovation Park, University of Pavol Jozef Šafárik in Košice, Department of Biophysics, Faculty of Science, University of Pavol Jozef Šafárik in Košice): **Purification of selected haloalkane dehalogenase evolved by ribosome display.**

17:30 - 17:50 **Zoltan Lipinszki** (Institute of Biochemistry, Biological Research Centre, Szeged, Hungary): **Challenges of producing recombinant viral antigens suitable for post-vaccination antibody testing**

Postranslational Modifications, Glycobiochemistry (Silver Lounge)

Ján Tkáč - chair
(Institute of Chemistry SAS, Bratislava)

16:30 - 16:50 **Veronika Vráblová** (Institute of Chemistry SAS, Bratislava): **Magnetic particles in bioaffinity interactions for implementation in the diagnostics of oncological diseases based on glycans.**

16:50 - 17:10 **Adam Paulin Urminský** (Research Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Brno, National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno)**: Paclitaxel-induced neuropathy: biomarker insights from glycoproteomics.**

17:10 - 17:30 **Michaela Mikysková** (Masaryk University, Brno): **Structural characterization of the jacalin-related lectin from the mushroom *Calocera viscosa***

17:30 - 17:50 **Drobnicov Memmorial 2023 - prize talk**

POSTER Session – 10.9-13.9.2023

18:00 - 19:00 **Poster discussion (The organizing committee will award three best posters at the FEBS3+ congress closing ceremony)**

The poster boards will be available for the entire time of the duration of the congress and will be located in the foyer of the Bellevue hotel.

Social event

Gala dinner 19:30 – 22:00

Tuesday, 12.9.2023

8:00 -18.30 **REGISTRATION /Foyer Hotel Bellevue/**

9:00 -9.50 **Plenary Lecture (Bellevue I)**

Marshall Bloom (National Institute of Alergy and Infection Diseases, USA): **New dimensions in the study of thick-borne virus infections.**

10:00 -11:30 **Lectures in parallel Sections**

Viruses, Bacteria, Protozoa & Parasites (Bellevue I)

Anton Horváth – chair
(Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava)

Libor Grubhoffer – chair
(Biology Centre CAS, České Budějovice)

10:00 – 10:30 **Vyacheslav Yurchenko** (University of Ostrava, Life Science Research Centre, Ostrava): **Catalase is detrimental for *Leishmania* virulence (with notes on evolution of catalases in *Trypanosomatidae*).**

10:30 – 10:45 **Ľubomíra Chmelová** (University of Ostrava, Life Science Research Centre, Ostrava): **Isocitrate dehydrogenases of trypanosomatids.**

10:45 – 11:00 **Ingrid Sveráková** (Department of Biochemistry, Faculty of Natural Sciences, Comenius University, Bratislava; Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice; Life Science Research Centre, Faculty of Science, University of Ostrava): **An unusual composition of mitochondrial pyruvate dehydrogenase complex of diplonemids, widespread marine protists.**

11:00 – 11:15 **Barbora Bučková** (Department of Biochemistry, Faculty of Natural Sciences, Comenius University, Bratislava): **Bioenergetics of marine protist *Paradiplonema papillatum*.**

11:15 – 11:30 **Gabriel Demo** (Central European Institute of Technology, Masaryk University, Brno; Department of Biology, Niigata University, Niigata, Japan): **Ribosome hibernation in *Archea.***

Proteomics (Bellevue II)

Peter Baráth- chair
(Institute of Chemistry SAS, Bratislava)
Jiří Dresler - chair
(Military Health Agency, Ministry of the Interior of the Czech Republic, Prague)

10:00 – 10:30 **Vladimír Havlíček** (Institute of Microbiology of the Czech Academy of Sciences, Prague): **Infectious site molecular mapping**

10:30 – 10:45 **Adam Pap** (Single Cell Omics Advanced Core Facility, Hungarian Centre of Excellence for Molecular Medicine, Laboratory of Proteomics Research, Biological Research Centre, Eotvos Lorand Research Network (ELKH) Szeged, Hungary): **Mass spectrometric characterozition of O-glycopeptides.**

10:45 – 11:00 **Marek Šebela (**Department of Biochemistry, Faculty of Science, Palacký University, Olomouc): **Enzyme activity assays by MALDI-TOF mass spectrometry.**

11:00 – 11:15 **Zuzana Kalaninová** (Faculty of Science, Charles University, Prague, BioCeV - Institute of Microbiology of the Czech Academy of Sciences, Vestec): **Proline semi-specific enzymes in the spotlight: The role of AnPEP in structural proteomics.**

11:15 – 11:30 **Maksym Danchenko** (Institute of Plant Genetics and Biotechnology - Plant Science and Biodiversity Centre, Institute of Chemistry, Slovak Academy of Sciences, Institute of Hydrobiology of National Academy of Sciences of Ukraine): **How do aquatic plants cope with pathogens in Chernobyl environment?**

11:30 -12:00 **Coffee Break**

12:00 -13:00 **Lectures in parallel Sections**

Viruses, Bacteria, Protozoa & Parasites (Bellevue I)

Anton Horváth – chair
(Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava)
Libor Grubhoffer – chair
(Biology Centre CAS, České Budějovice)

12:00 – 12:15 **Hana Drahovská** (Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava): **Characterization of bacteriophages infecting *E. coli* and *Cronobacter*.**

12:15 – 12:30 **Monika Záhorszká** (Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava): **Pretomanid and delamanid as the newest players against tuberculosis.**

12:30 – 12:45 **Kristína Záhonová** (Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Vestec, Czech Republic): **Comparative analysis of mitochondrion-related organelles in anaerobic amoebozoans.**

12:45 – 13:00 **Dušan Žitňan** (Institute of Zoology, SAV, Bratislava, Slovakia): **Expression and function of specific neuropeptide-receptor pathways in ticks**.

Companies´ presentations (Silver Lounge)

12:00 - 12:20 **Balint Nagy** (Editor-in-Chief of journal MCP, Elsevier) and **Viorica Liebe Lastun** (Scientific Editor at Elsevier LTD): **How to successfully publish in MCP—Advancing Biology and Medicine through Omics and Bioinformatic** (Elsevier prize for four best lectures by young scientists in sections: Molecular Basis of Disease and Therapy; Regulation of Gene Expression, Epigenetics; Biotechnology And Technology Transfer In Life Sciences; DNA Sequencing – Methods and Applications).

12:20 - 12:40 **Kateřina Kolková** (SPECION, s.r.o): **Modern Methods for Life Science Applications.**

13.00 -14.30 **Lunch**

14:30 -16:00 Lectures in parallel Sections

Biotechnology and Technology Transfer in Life Sciences **(Kristal Lounge)**
Pavol Miškovský - chair
(Cassovia New Industry Cluster, Košice)
Erik Sedlák - chair
(Technology and Innovation Park of the Pavol Jozef Šafárik University in Košice)

14:30 - 15:00 **Pavol Miškovský** (Cassovia New Industry Cluster, Košice): **Application of nanotechnology-based sensors in environment, medicine and food control.**

15:00 - 15:15 **Martin Humenik (**Department of Biomaterials, Faculty of Engineering Science, University Bayreuth, Bayreuth): **Recombinant spider silk-DNA hybrids in materials engineering.**

15:15 - 15:30 **Ondrej Pös (**Comenius University Science Park, Bratislava, Slovakia; Geneton Ltd., Bratislava): **The potential of liquid biopsy in the era of precision medicine.**

15:30 - 15:45 **Erik Sedlák (**Center for Interdisciplinary Biosciences, Technology and Innovation Park, Pavol Jozef Šafárik University Košice, Department of Biochemisty, Faculty of Science, P.J. Šafárik University): **Alternative design of flavoprotein-based photosensitizers.**

15:45 - 16:00 **Ruta Lavinia Liliana (**University of Bucharest, Faculty of Chemistry): **Yeast surface display platform for the selection of GFP and mCherry nanobodies**.

Regulation of Gene Expression, Epigenetics (Bellevue II)

Andrea Šoltýsová - chair
(Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava)
Lucia Messingerová - chair
(Centre of Biosciences SAS)

14:30 - 15:00 **Aladar Pettko-Szandtner** (Laboratory of Proteomics Research, Biological Research Centre, Szeged, Institute of Plant Biology, Biological Research Centre, Szeged, Single Cell Omics Advanced Core Facility, Hungarian Centre of Excellence for Molecular Medicine, Szeged): **Proteomic stories/Determining the phosphorylation map of plant retinoblastoma-related (RBR)protein in proliferating tissues.**

15:00 - 15:20 **Jiří Vrba** (Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Olomouc): ***In vitro* systems for investigating metabolic sulfation of natural polyphenols.**

15:20 - 15:40 **Agnes Tantos** (Research Centre for Natural Sciences, Budapest): **Regulatory roles of RNA binding in histone methyltransferases.**

15:40 - 16:00 **Andrea Šoltýsová** (Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava): **Epigenetic landscape in prognosis of uveal melanoma.**

16:00 -16:30 **Coffee Break**

16:30 -17:30 **Lectures in parallel Sections**

Biological membranes (Kristal Lounge)

Peter Račay - chair
(Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University in Bratislava)
Lucia Balážová - chair
(BMC SAS, Bratislava, Institute of Food, Nutrition and Health, ETH Zurich)

16:30 – 17:15 **Christos Chinopoulos** (Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary): **Non-oxidative phosphorylation energy-harnessing pathways in respiration-impaired mitochondria.** - invited speaker

17:15 – 17:35 **Lucia Balážová** (BMC SAS, Bratislava, Institute of Food, Nutrition and Health, ETH Zurich): **Targeting GPR180 modulates glucose homeostasis by enhancing energy expenditure and insuline secretion.**

17:35– 17:55 **Peter Račay** (Comenius University in Bratislava, Jessenius Faculty of Medicine): **To be under stress, or not to be under stress, that is the question.**

DNA sequencing – methods and applications/Bioinformatics (Computational Biochemistry (Bellevue II)

Tomáš Szemes - chair
(Department of Molecular Biology, Faculty of Natural Sciences, Science Park, Comenius University in Bratislava)
Jaroslav Budiš - chair
(Science Park, Comenius University in Bratislava)

16:30 – 17:00 **Bálint Nagy** (University of Debrecen, Faculty of Medicine, Department of Human Genetics, Hungary): **Clinical application of cell-free nucleic acids. - invited speaker**

17:00 – 17:30 **Vladimír Beneš** (EMBL Heidelberg)**: My forty DNA sequencing years.**

17:30 – 17:45 **Tomáš Szemes** (Department of Molecular Biology, Faculty of Natural Sciences, Science Park, Comenius University in Bratislava): **Narcos and Beyond: National Surveillance of SARS-CoV-2 and Other Pathogens.**

17:45 – 18:00 **Ľubica Urbániková** (Institute of Molecular Biology, SAS): **Family GH13 trehalose synthases with a C-terminal maltokinase – an *in silico* study.**

18:00 – 18:15 **Matej Hrnčiar** (Geneton s.r.o): **Identifying Microsatellite Instability through Analysis of Sequencing Data**

18:15 – 18:35 **Mariana Šatrová, Ondřej Pácalt** (GeneTiCA, s.r.o.): **Squeeze the genome with long reads**

18:35 – 18:55 **Dmytro Omelchenko** (application specialist at Dynex, s.r.o.): **Sequencing by avidity: unprecedented data quality at affordable price.**

Social event

19.30 - wine tasting

Wednesday 13.9. 2023

FEBS special Session on Research and Career skils **(Bellevue I)**
09:00 – 09:30 **Václav Pačes** (Institute of Molecular Genetics, Czech Academy of Science, Praha):

Teaching in Biochemistry, Biological Chemistry and Molecular Biology study programs **(Bellevue I)**
09:30 – 10:30
Mária Mareková - chair
(Medical University, Pavol Jozef Šafárik University in Košice)
Ján Lehotský - chair
(Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University in Bratislava)

09:30 – 09:40 **Mária Kožurková** (Department of Biochemistry, Institute of Chemical Sciences, Faculty of Science, Pavol Jozef Šafárik University in Košice): **Post-graduate study program in Biochemistry at the Faculty of Sciences of Pavol Jozef Šafárik University in Košice.**

09:40 - 09:50 **Mária Mareková** (Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice): **How is changing role of chemical subjects in medical study curriculum last decade?**

09:50 - 10:00 **Boris Lakatoš** (Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava)**: Teaching of biochemistry at FCHPT STU – impact of new accreditation, COVID-19 and the project "ACCORD".**

10:00 - 10:10 **Stanislav Stuchlík** (Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava): **Bioscience study programs at the Faculty of Natural Sciences CU and Department of Molecular Biology.**

10:10 - 10:20 **Jiří Hudeček** (Department of Biochemistry, Faculty of Science, Charles University, Prague): **FEBS Educational Ambassadors**

10:20 - 11:00 **Coffee Break**

Workshop: Women in science (Gold Lounge)

Zuzana Staňáková - chair
(Slovak Centre of Scientific and Technical Information, Bratislava)
Jana Kottulová - chair
(Faculty of Arts, Comenius University, Bratislava)

11:00 - 11:20 **Zuzana Staňáková** (Slovak Centre of Scientific and Technical Information, Bratislava): **Inclusive Gender Equality in the Life Sciences. Importance of Using the Full Pool of Talents in Biochemistry**.

11:20 - 11:40 **Jana Kottulová** (Faculty of Arts, Comenius University, Bratislava): **Why should we care about gender equality in science?**

11:40 - 12:00 **Danica Zendulková** (Slovak Centre of Scientific and Technical Information, Bratislava): **Women in Slovak Life Sciences Projects.**

12:00 **Closing ceremony (Bellevue I):**

- **Poster prize winners announcement**
- **Elsevier prize winners announcement**
- **Lecture prize winners announcement** (Information about BIOTECH, IAB, GeneTiCA - prize for three best lectures by young scientists in four sections: Recombinant Protein Production; DNA Sequencing – Methods and Applications; Proteomics)

13:00 **Lunch**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NEW DIMENSIONS IN THE STUDY OF TICK-BORNE VIRUS INFECTIONS

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Abstract: Recent years have seen the emergence of new tick-borne viruses in several virus families around the world; including severe fever and thrombocytopenia syndrome virus, Heartland virus and Bourbon virus. At the same time, a number of other well-known but worrisome tick-borne viruses continue to re-emerge, notably tick-borne encephalitis virus, Kyasanur Forest disease virus and Powassan/deer tick virus, and Congo-Crimean hemorrhagic fever virus. As a consequence, there has been renewed interest in the study of these agents, and a recognition that a multi-dimensional analysis must consider the virus, the tick and the mammalian host. We have applied this comprehensive strategy to the tick-borne flaviviruses (TBFV) - the BSL-3 Powassan/deer tick virus (POWV/DTV) and the BSL-2 Langat virus (LGTV). Molecular virology, imaging, in situ hybridization, genomics, and genome wide screens are being employed to examine acute and persistent LGTV infection in vitro and in vivo. *Ex vivo* culture and artificial membrane feeding systems have enabled tightly controlled characterization of POWV/DTV and LGTV infection in the tick target organs and the demonstration that silencing tick genes can impact impact virus replication. Finally, *in vivo* studies have been used to elucidate mechanisms of chronic disease following TBFV infections. The combined approach has yielded new insights into these infections, applicable to other tick-borne virus infections.

This work was supported by the Intramural Program of NIH/NIAID.

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CANNABINOIDS AS MODULATORS OF CALCIUM SIGNALING AND INFLAMMATION

ANDREA FLEIG

Center for Biomedical Research at The Queen's Medical Center and University of Hawaii Cancer Center and John A. Burns School of Medicine, Honolulu, USA

Abstract: Cannabis sativa has long been known to affect numerous biological activities. Plant extracts, purified cannabinoids, or synthetic cannabinoid analogs have shown therapeutic potential in pain, inflammation, seizure disorders, appetite stimulation, muscle spasticity, and treatment of nausea/vomiting. This presentation provides a comprehensive overview of the effects of whole-plant Cannabis extracts and various pure cannabinoids on storeoperated calcium (Ca2+) entry (SOCE) in several different immune cell lines [1]. Store-operated Ca2+ entry is one of the most significant Ca2+ influx mechanisms in immune cells. Carboxylic acid derivatives, and particularly cannabigerolic acid (CBGA), demonstrates high potency against SOCE by blocking calcium release-activated calcium (CRAC) currents. CBGA is also highly effective in ameliorating inflammation and fibrosis in a cisplatin mouse model, holding promise as a kidney-protective adjuvant during cancer treatment [2]. Our results indicate that cannabinoid-mediated inhibition of a proinflammatory target such as SOCE may at least partially explain the anti-inflammatory and analgesic effects of Cannabis.

1. Faouzi M, Wakano C, Monteilh-Zoller MK, Neupane RP, Starkus JG, Neupane JB, Cullen AJ, Johnson BE, Fleig A, Penner R (2022). Acidic cannabinoids suppress pro-inflammatory cytokine release by blocking Store-Operated Calcium Entry. FUNCTION 3(4):zqac033. DOI: 101093/function/zqac033.
2. Suzuki S, Fleig A, Penner R (2023) CBGA ameliorates inflammation and fibrosis in nephropathy. Sci Rep 13(1):6341. DOI: 10.1038/s41598-023-33507-2.

Keywords: Drug Development, Cannabinoids, CRAC, SOC

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RESTRICT CANCER METASTASIS - SAVE PATIENT LIFE: TRANSLATING GENE DISCOVERY INTO CLINICAL APPLICATION

ULRIKE STEIN

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Charité - Universitätsmedizin Berlin, and Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Abstract: Cancer metastasis is the most lethal attribute of cancer. It is responsible for >90% of cancer deaths, still remaining a major treatment challenge. Metastatic dissemination of primary tumors critically limits successful therapy in many tumor entities and is directly linked to patient survival. Thus, Metastasis-associated biomarkers identifying cancer patients at high risk for metastasis and shortened survival and simultaneously acting as key drivers for metastasis are extremely desired. Clinical interventions targeting these molecules are of highest importance.
In search of drivers of metastasis we identified the novel, previously undescribed gene Metastasis-Associated in Colon Cancer 1 (MACC1) in human colorectal cancer (CRC). MACC1 induces fundamental processes like cell proliferation, migration, invasiveness and metastasis in xenografted and transgenic mice. Meanwhile, MACC1 has been established by us and others as key player, prognostic and predictive biomarker for tumor progression and metastasis in >20 solid cancers. Meanwhile, >320 MACC1 papers (PubMed) from groups worldwide were published until today, incl. meta-analyses on solid cancers.
In recent years, we discovered regulating and regulated networks of metastasis genes, and their functional impact in cell culture, animal models, patient tumor tissue and blood. We unveiled transcriptional targets, protein-protein interactors, and post-translational effectors, serving as new diagnostic, prognostic and predictive key players for tumor progression and metastasis. We identified repositioned drugs and novel compounds as transcriptional and post-translational small molecule inhibitors, restricting MACC1-induced metastasis in mice.
Together with the metastasis inducer S100A4, which we first identified as Wnt-signaling target and metastasis predictor, but also as a MACC1 transcriptional target, we demonstrated their beneficial combinatorial impact for early identification of high-risk cancer patients. We exploited this knowledge for improved prognosis and response prediction using tumors and blood of solid cancer patients as well as for combinatorial therapies.
Key drivers of metastasis, such as MACC1 and S100A4, their regulation and role in cancer cell signaling, their functional impact in metastasis formation, their contribution for solid cancer metastasis patients (tumor tissue and blood) and novel anti-metastatic therapy options represent the focus of our research. All these endeavors are performed in translational approaches by bridging our experimental data to their clinical relevance and clinical use. The ultimate goal is the signaling-based establishment of new therapeutic concepts for metastasis restriction and prevention. Novel combinatorial therapeutic approaches are tested in clinical trials to treat patients with metastatic disease using newly identified repositioned small molecule inhibitors acting on these biomarkers.

Keywords: cancer metastasis, MACC1, signaling network, clinical impact, intervention

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RNA BINDING PROFILES OF TWO HISTONE LYSINE METHYLTRANSFERASES

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Abstract: Histone lysine methyltransferases (HKMTs) perform vital roles in cellular life by controlling gene expression programs through the posttranslational modification of histone tails, giving the understanding of the molecular mechanisms that control their target recognition and activity high scientific importance.
Members of the KMT2 (or MLL) family are responsible for the mono- and di-methylation of histone H3 lysine 4 (H3K4) in the enhancer and promoter regions of actively transcribed genes. In yeast, all H3K4 methylation events are carried out by a single Set1 protein, while in *Drosophila*, there are responsible for H3K4 methylation. In mammals, two orthologs are found for each *Drosophila* H3K4 methyltransferase, KMT2A and B (MLL1/2), KMT2C and D (MLL3/4) and KMT2E and F (SETD1A/B). These pairs have largely, but not completely overlapping functions; indicating the relevance of roles different from histone methylation.
Recently, RNA binding has been shown to be an important feature of several HKMTs, even for those that lack canonical RNA binding domains. Nevertheless, the generality and the details of these mechanisms remain largely uncharted indicating the need for directed studies.
To broaden our understanding of the involvement of RNA binding in the functions of KMT2 proteins, we studied two family members, KMT2D and KMT2F. While a canonical RRM domain can be found in KMT2F, KMT2D only contains non-canonical, predicted RNA binding regions. Using RNA immunoprecipitation, we identified a broad range of coding and non-coding RNAs associated with both proteins and in addition to the confirmation of the in cell RNA-binding, we could show *in vitro* RNA interaction for the RNA interacting elements in both proteins.
Analysis of the bound mRNAs revealed that KMT2D and KMT2F interact with a largely non-overlapping set of RNAs within the nucleus, indicating different regulatory roles for the RNA binding in these proteins.

Keywords: KMT2D, KMT2F, Histone lysine methyltransferase, RIP-sequencing, RNA binding

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CHARACTERIZATION OF BACTERIOPHAGES INFECTING *E. COLI* AND *CRONOBACTER*

ANDREZÁL MICHAL[1], MARKUSKOVÁ BARBORA[2], SULAFA ELNWRANI[1], SZEMES TOMÁŠ[1,2], KAJSIK MICHAL[1,2], DRAHOVSKÁ HANA[1,2]

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Abstract: One of the most critical challenges of contemporary medicine is the continual spread of new antimicrobial resistance mechanisms among important human bacterial pathogens. Presently, the demand for alternative therapies for difficult-to-treat infections, including biofilm associated infections, is steadily rising. Phage therapy as a promising way to achieve this aim is now experiencing its renaissance. This procedure requires well-defined virulent phages which attack pathogenic bacteria in a strain specific manner. Multiplication of therapeutic phages at the site of infection increases the efficiency of bacterial clearance. All this happens without adverse effects on the equilibrium of microbiota or toxicity to the human body and other usual side-effects of antibiotic treatment. Moreover, phages are affected neither by mechanisms of antimicrobial resistance nor by drug interactions, which are common in antibiotic therapy.
Escherichia coli, a member of the family *Enterobacteriaceae*, is a commensal gut bacterium as well as an opportunistic pathogen causing both extra intestinal and intestinal pathologies. It is the most common causative agent of urinary tract infections and *E. coli* ST131 clone resistant to antibiotics represents a big problem. *Cronobacter* and *Enterobacter* are other enterobacterial opportunistic pathogens capable of producing a wide variety of infections.
In the present study we isolated and characterized bacteriophages with broad host specificity against a panel of local *E. coli*, *Cronobacter* and *Enterobacter* strains for the establishment of a national phage bank. Sixteen different bacteriophages infecting UPEC strains were isolated from the sewage and surface water. By host specificity determination they were able to infect 2 - 44 (3 - 57%) strains, 80% strains were infected at least by one phage. Susceptible *E. coli* strains belonged predominantly to the B2 phylogenetic group including strains of two worldwide spread clones CC131 and CC73. All phages belonged to class *Caudoviricetes*. Majority of them were classified into family *Straboviridae* (T4 like phages), *Autographiviridae* (n=2) and *Drexlerviridae* (n=2) and genera *Kagunavirus* (n=2), *Justusliebigvirus* (n=1) and *Murrayvirus* (n=1). Six phages with the best properties were combined into a phage cocktail and its antibacterial activity was measured in liquid artificial urine medium. Bacteriophages infecting *Cronobacter* strains belonged to predominantly *Straboviridae* and *Autographiviridae* families. They proved to be useful in prevention of *Cronobacter* growth in rehydrated powdered infant formula.

Keywords: phage therapy, bacteria, infection resistance

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ROLE OF MAGNESIUM IN ENERGY AND CALCIUM HOMEOSTASIS OF DAIRY COWS - TRANSLATIONS TO HUMANS

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Abstract: Homeostasis of magnesium (Mg) is crucial for several key metabolic functions given the role of Mg in the regulation of neuronal excitability, ATP homeostasis and hundreds of enzymatic reactions. Blood plasma Mg concentrations result from gastrointestinal absorption, cellular uptake, bone deposition and urinary excretion. In contrast to other cationic macrominerals, there is no dedicated hormonal control of Mg homeostasis albeit it is co-regulated with metabolic (insulin) and calcium balance signals (parathyroid hormone). However, the latter co-regulation can be counterproductive to some extent. For example, Mg deficiency decreases the effects of parathyroid hormone, which can further aggravate Mg deficiency due to decreased release of Mg from bone. In the absence of efficient hormonal regulation, Mg fluxes appear to be dependent on concentration and electrical gradients, as well as the local expression and activity of Mg-responsive genes.

Magnesium availability for herbivores differs substantially from that in omnivorous or carnivorous species because diets based on fibrous plant material contain relatively high concentrations of Mg. Despite this fact, herbivores like dairy cows may suffer from even severe Mg deficiency that may proceed to clinical "grass" tetany. This Mg deficiency is due to a diet high in potassium that interferes with Mg absorption, which is further exacerbated by low sodium and high protein contents of the diet.

Although hypomagnesaemic tetany has long been the primary focus of Mg research, there is growing recognition that other ailments may also be associated with disturbances in Mg homeostasis. As mentioned above, low plasma Mg concentration interferes with parathyroid hormone signalling and thus may be a contributing factor to the very common postparturient hypocalcaemia in dairy herds. Recent results from our laboratory indicate that postparturient hypocalcaemia may be additionally promoted by local vitamin D-independent effects of low Mg concentration on osteoblasts, leading to enforced bone mineral deposition. These findings may be highly relevant to humans, explaining disturbances in calcium homeostasis during hypomagnesaemia and disturbances in bone healing when Mg alloys are used in surgical bone fixation. Vice versa, there is emerging evidence that the effects of low Mg on metabolic syndrome in humans may also be partly applicable to the postparturient metabolic syndrome in dairy cows. Comparing the effects of Mg on the highly different metabolism of glucose and lipids in humans vs. ruminants may lead to a better understanding of the general principles of the role of Mg in energy and mineral homeostasis.

Keywords: Bone metabolism, Calcium, Dairy cow, Magnesium, Metabolic syndrome

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CHALLENGES OF PRODUCING RECOMBINANT VIRAL ANTIGENS SUITABLE FOR POST-VACCINATION ANTIBODY TESTING

EDIT ÁBRAHÁM[1,2], ANNAMÁRIA MARTON[2], CSABA BAJUSZ[2], NORBERT PARDI[3], ZOLTÁN LIPINSZKI[1,2]

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Abstract:
The development of recombinant DNA technology in the early 1970's has revolutionised all aspects of biology and opened new avenues for basic and applied sciences. With this technique, protein-encoding genes can be engineered and expressed in various heterologous systems (including bacteria, yeast, insect, or mammalian cells) to produce and purify active and properly folded proteins of interest. This includes polypeptides for industrial, agricultural, therapeutic, diagnostic, as well as basic research purposes. Recent emergence of the new generation mRNA-LNP (lipid nanoparticle)-based vaccines, which are developed against viral or parasite infections, or tumour progression, represented new challenges in recombinant protein (antigen) production, as well. Although mRNA-LNP vaccines bypass the need for high purity protein antigens to be used in the immunisation process, which is one of their main advantages over classical vaccines, the potency of vaccination, and the development of cellular immunity, is often tested by serological assay (e.g., ELISA, ELISpot, etc.). This, however, requires soluble and properly folded proteins or protein fragments/domains. We design, express, and purify various viral proteins for serological assay to validate and test the efficiency of vaccines against viruses, including SARS-CoV-2, influenza virus, or African Swine Fever Virus (ASFV). While some of these antigens can be expressed in bacteria, others require homo- or heteromerization, co-expression with their chaperones or partners, or specific post-synthetic modifications for their active structure, which is often tricky and require optimisation at various levels. I will present a few examples of how we have overcome these problems and demonstrate the challenging production of ASFV antigens that we successful used in ELISA and ELISpot experiments.

This work was supported by the National Laboratory for Biotechnology (2022-2.1.1-NL-2022-00008) to CSB and ZL, and the Hungarian Academy of Sciences (Lendület Program Grant (LP2017-7/2017)) to ZL.

Keywords: recombinant protein, protein purification, antigen design, mRNA-LNP vaccines, viral antigens, ASFV

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TARGETING GPR180 MODULATES GLUCOSE HOMEOSTASIS BY ENHANCING ENERGY EXPENDITURE AND INSULIN SECRETION

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Abstract: Half of the adult population will be overweight or obese by 2035. Obesity is a complex metabolic disorder associated with many comorbidities such as type 2 diabetes, fatty liver and cardiovascular diseases as well as cancer. Effective therapeutic strategy to combat obesity must target food intake, energy expenditure or both. Drugs reducing food intake often cause nausea and gastrointestinal complications. On the other hand, increasing energy expenditure via lifestyle modification is unfortunately not sustainable on the long term in majority of the population with obesity. As an alternative, stimulation of adipose tissue thermogenesis is an appealing strategy to burn excessive energy in obesity.

Recently, we discovered a so far orphan receptor GPR180 to be enriched in human thermogenic brown adipose tissue. Utilizing a set of gain and loss of function mouse and in vitro studies we found that this receptor mediates the action of hormone CTHRC1 to increase metabolic rate of adipocytes and thereby contribute to enhanced glucose utilization. Mechanistically, we showed that GPR180 was incorrectly annotated to G protein coupled receptor family and it integrates to TGFb signalling pathway, while fine-tuning its activation. Importantly, we demonstrated association of circulating CTHRC1 in human with improved metabolic profile. Interestingly, using a genetic model with inducible adipocyte-specific ablation of GPR180, we found that loss of this receptor in adipocytes does not fully recapitulate the phenotype observed in global knockout mice indicating important role of GPR180 in another organ/tissue in regulation of glucose homeostasis. Pancreatic beta cell specific deletion of GPR180 revealed a defective glucose stimulated insulin secretion in the knockout mice without altering percentage of insulin producing cells. By the use of MIN6 cell line with beta cell characteristics, we currently explore the mechanism by which GPR180 affects insulin secretion.

It seems that GPR180 represents a promising target to develop new drugs to combat metabolic disturbances at multiple levels – by enhancing energy expenditure via adipocyte thermogenesis and pancreatic insulin secretion.

This work is supported by grants SASPRO2 No.1260/02/02 (European Union´s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 945478).and grant VEGA no. 2/0128/23.

Keywords: adipocyte thermogenesis, pancreatic beta cells, insulin release

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MY FORTY DNA SEQUENCING YEARS.

VLADIMÍR BENEŠ

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Abstract: Introduction of DNA sequencing into the armamentarium of biochemical methods in the late seventies of the last century was very timely and important for an advancement of recombinant DNA technology. It is because it provided, and it certainly continues to do so, an opportunity to detect precisely all modifications, intended as well as unintended…
Over the years, thanks to many technical developments and refinements of hardware, enzymes and other reagents as well as computational solutions, this method has evolved into the robust and powerful analytical technique enabling almost bias-free exploration of the complete nucleic-acid space across the large sample scale.
I will share my first-hand experience from my modest beginnings with this method 40 years ago until its 'massively parallel sequencing' stage today.

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BIOENERGETICS OF MARINE PROTIST *PARADIPLONEMA PAPILLATUM*

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Abstract: Diplonemids are a group of heterotrophic unicellular organisms that belong to the phylum Euglenozoa, including diplonemids, kinetoplastids, euglenids, and symbiontids. Diplonemids have recently been shown to be one of the most numerous and diverse groups of protists inhabiting the marine ecosystem. They occur naturally in different areas of the oceans where they are forced to adapt to changing conditions such as nutrient and oxygen availability. Transcriptome analysis shows that the metabolism of *Paradiplonema papillatum*, a diplonemid model organism, is flexible enough to respond to these changes, possessing transcripts for enzymes of both aerobic and anaerobic metabolism. Here, we investigate the functional involvement of individual bioenergetic pathways in various conditions that paradiplonema can face. An oxygen uptake study revealed mitochondrial respiration sensitive to potassium cyanide. By direct measurement of ATP production, we confirmed the connection between respiration and ATP synthesis. We also show that despite not regulating its anaerobic metabolism, *P. papillatum* is able to survive in the absence of oxygen and even generate ATP. Based on our results, we proposed a bioenergetic pathway that could be active in *P. papillatum* under individual culture conditions.
Supported by grants SK-CZ-RD-21-0038 and VEGA 1/0553/21

Keywords: *Paradiplonema papillatum*, hypoxia, normoxia, oxidative phosphorylation, ATP

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PURIFICATION OF SELECTED HALOALKANE DEHALOGENASE EVOLVED BY RIBOSOME DISPLAY

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Abstract: Haloalkane dehalogenases (HLDs) are hydrolytic enzymes cleaving carbon-halogen bonds resulting in the formation of primary alcohols, halide ions and protons. HLDs potential lay in their ability in decontamination and bioremediation of environment. However, the bottleneck of HLDs broader utilization is their low affinity and specificity for certain substrates. To address these issues, we applied a protein evolution approach represented by ribosome display method. The outcome of protein selection by this method is numerous variants of proteins with various conformational and functional properties. The aim of this work is the production of selected variant of DhaA, D15, in bacterial expression strain BL21, purification, refolding and a basic biophysical characterization of the variant.

Keywords: haloalkane dehalogenase, ribosome display, protein purification, DhaA

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HOLISTIC ANALYSIS OF GENE EXPRESSION (NGS, PROTEOMICS) AND FUNCTIONS IN ANIMAL DISEASES: CANINE CANCER AND VARROA TREATMENT IN HONEYBEES AS EXAMPLES

RALF EINSPANIER, TANIA GUTIERREZ-RIQUELME, ANTONIA GENATH, MORITZ MATING, TORSTEN STEIN

Institute of Veterinary Biochemistry, Freie Universitaet Berlin, Germany

Abstract: Specific molecular analysis of serious diseases in veterinary medicine is particularly ambitious because there are many different animal species, most of which have different metabolic pathways and whose genome or proteome is rarely fully available. In order to diagnose and subsequently treat important diseases in the diverse species (most interesting are livestock and companion animals), appropriate samples paired with modern molecular biological analysis techniques should be necessary. Here, two animal systems will be introduced as examples for elucidating first molecular basis for dysregulated cellular signaling cascades: First, (I) selected canine cancer cell lines secreting extracellular vesicles (EV) are monitored for specifically regulated protein abundance compared to controls. Dysregulated candidate genes found in EVs may possess the potential as further diagnostic tools. Second, (II) a project is presented deciphering the molecular mode of action of formic acid (FA), a common treatment against the honeybee parasite, Varroa destructor. The identification of potentially involved insect enzyme systems may thus contribute to a better understanding of the mode of action as well as to a more optimized FA-treatment in the future. The aims of these two case studies were to detect new target molecules (biomarker), by (I) searching for EV-based peripheral target molecules to diagnose dog cancer in early stages, and (II) to clarify the mode of action of the frequently used varroazide formic acid (FA). These findings should improve the intended cellular effects and the development of more specific therapies in both species. By use of biochemical (cell culture, EV isolation and characterization, 2D electrophorese and/or mass spectroscopy, enzyme activity measurement, computational protein modelling) as well as molecular genetic methods (RT-qPCR, RNAseq, miRNA, RNAi, recombinant protein expression) first insights in differently regulated disease-/pathogen-related genes and proteins were enabled. Based on modern molecular methods combined with computational pathways analysis, we were able to select significantly regulated transcripts and proteins in both animal systems. Significantly regulated candidate gene products were identified and verified for the first time in dogs and honeybees. In conclusion, our research will (I) provide novel EV-based molecular biomarkers for selected cancer cells in dogs that may be useful for cancer diagnosis and therapy. Further basic results should (II) allow us to better understand the molecular mechanisms of FA treatment against Varroa in honeybees enabling more efficient treatment strategies less harmful to honeybees. This knowledge of new effector molecules will be of great benefit for both diagnostics and therapy in canine cancers or threats to honeybee health.

Keywords: canine cancer, honeybee, varroa, RNAseq, proteomic

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HEXATRIACONTAPEPTIDES DESIGNED AS METAL-BINDING TAGS

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Abstract: Genetically modifying proteins with various peptide tags significantly contributed to protein functional studies, to high and rapid purification of recombinant proteins, or to conferring a target protein new characteristics. In this study we designed two hexatriacontahexapeptide tags with potential metal-binding traits. The peptides, formed of thirty-six amino acids, had the primary sequence LM**E**QL**EE***C*HQ**E**H**EE**CGPGNG**EECE**Q*C*H**EE**HMQLM**E**L (Seq1) and LM**E**QL**EE***M*HQ**E**H**EE**CGPGNG**EECE**Q*M*H**EE**HMQLM**E**L (Seq2). Seq2 differed from Seq1 at positions 8 and 26 (italics), where cysteine was replaced with methionine. Secondary structure prediction indicated that the two peptides form a coil-coil motif, with the 12 negatively charged glutamyl residues (E) facing outwards and the four histidyl residues (H, underlined) facing the space between the helices. Such a secondary structure would allow E to bind non-specifically any positively charged metal ion, while the four H facing inward would be stabilized by imidazolyl-phylic cations, such as Co(II) or the paramagnetic Ni(II). Using successive PCR we introduced artificial DNA sequences encoding Seq1 and Seq2 at the *C*-terminus of myrGFP (Green Fluorescent Protein bearing a myristoylation sequence) cDNA, and the constructs were further expressed in *Saccharomyces cerevisiae* cells as myrGFP-Seq1/2. The *myrGFP* casette introduces a myristoylation site, allowing both directional targeting to the inner face of the plasma membrane and monitoring of the intracellular localization of the construct. To estimate and to control the potential toxicity of the constructs, the expression of *myrGFP-Seq1/2* was monitored by placing the chimeric DNA under the inducible *GAL1* yeast promoter. The constructs were investigated against an array of heavy metals in terms of metabolic changes, growth defects and heavy metal (hyper)accumulation. It is expected that the hexa-triacontahexapeptide tags designed would confer a recombinant protein new traits, depending on the metal targeted. Such tags are promising tools on the way to obtain cell factories for organic-framed magnets or cloneable nanoparticles.

Keywords: Hexatriacontapeptide, *Saccharomyces cerevisiae*, metal-binding sequence, GFP

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ALTERNATIVE DESIGN OF FLAVOPROTEIN-BASED PHOTOSENSITIZERS

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Abstract: Flavin mononucleotide (FMN) is a common cofactor consisting of a heteroaromatic isoalloxazine ring, which provides a basis for the broad chemical versatility of flavoenzymes. The isoalloxazine ring in FMN also belongs to efficient endogenous photosensitizers producing singlet oxygen, $^1O_2$, with the potential of its utilization in photodynamic therapy. FMN as a small organic compound has limited ability to be targeted, but as a part of genetically-encoded photosensitizers, it can be achieved using various tags. However, FMN encompassed in protein has significantly diminished the efficiency of $^1O_2$ production due to its interactions with surrounding amino acid residues. Recently, an increase of $^1O_2$ production yield by FMN buried in a protein matrix was achieved by a decrease in quenching of the cofactor excited states by weakening the protein-FMN interactions while still forming a complex. We suggest an alternative approach, which relies on light irradiation-induced dissociation of FMN to solvent. Such dissociation unlocks the full capacity of FMN as a $^1O_2$ producer. Our suggestion is based on the study of an irradiation effect on two variants of the LOV2 domain from *Avena sativa*; wild type, AsLOV2 wt, and its variant with a replaced cysteine residue, AsLOV2 C450A [1]. This hypothesis has been tested by a formation of several mutants, namely, two single mutants: V416C and T418C, and one double mutant: V416C/T418C. Our analyses of the proteins before and after irradiation of the proteins by numerous experimental methods, such as absorbance spectroscopy, fluorescence, circular dichroism, and differential scanning calorimetry as well as by theoretical *in silico* approaches showed the irradiation-induced conformational changes and oxidation of several amino acids. More importantly, a detailed analysis of these observations indicates that irradiation-induced increase in $^1O_2$ production is proportional to a release of FMN from the protein. This result indicates an approach to the production of more efficient photosensitizers by a design of isoalloxazine ring-binding site in flavoproteins.

Keywords: flavoproteins, photosensitizers, singlet oxygen, rational design

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RECOMBINANT SPIDER SILK-DNA HYBRIDS IN MATERIALS ENGINEERING

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Abstract: We created DNA-spider silk conjugates by coupling short DNA strands with the recombinant protein eADF4(C16) using click chemistry. The resulting hybrid materials had a spider silk moiety that enabled self-assembly into nanofibrils and a DNA part that allowed for sequence-specific labeling (1) or immobilization (2) of the conjugates. Recently, we used these hybrids to create immobilized fibril-based networks that were functionalized with aptamers. These networks exhibited swelling and softening properties and served as protective depots for enzymes (3). In addition, we used the DNA-functionalization and cell-repellent properties of the networks to develop specific cell immobilization strategies. We introduced a mild lipid-DNA modification of cell membranes, which showed high labelling efficiency and negligible cytotoxicity. By taking advantage of the complementarity of nucleic acids, we achieved highly specific DNA-assisted immobilization of cells on spider silk nanohydrogels with tuneable cell densities. Competitor DNA probes allowed us to lift off the cells on demand (4). Furthermore, we found that nanohydrogels containing aptamers could bind specifically to cell markers and immobilize different types of cancer cells (5). We also combined the principle of surface nucleated fibril self-assembly and nanohydrogel formation with photolithography. By patterning a positive-tone resist on an amino-reactive substrate, we created microwells of arbitrary shapes. The bottoms of these microwells defined the binding of spider silk nucleation sites and the formation of fibrillar networks (6). After stripping the sacrificial photoresists, we achieved microstructured patterns of nanohydrogels that enabled DNA-assisted immobilization of DNA-labeled cells (4) or aptamer-cancer cell marker interactions with high spatial fidelity (5).

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RIBOSOME HIBERNATION IN ARCHEA

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Abstract: Under stress conditions in bacterial cells, the formation of a hibernating ribosome dimer (termed 100S)[1-3], is a useful adaptation mechanism, which results in suppression of protein synthesis in the stationary phase. In *E. coli*, two protein factors, RMF and HPF, are involved in regulation of dimerization of the 70S ribosome[1-3]. RMF directly participates in dimerization of 70S ribosomes and HPF stabilizes the 100S ribosomes. However, homologous proteins of RMF and HPF have not been found in archaea. Therefore, further investigations of the molecular mechanisms of ribosome hibernation in archaea are required.
Here, we present a single particle cryo-EM study, revealing a novel ribosome dimerization factor (RDF) in archea. The RDF is highly specific to archeal ribosomes and is capable to interact only with archeal 30S small ribosomal subunit[4]. In order to determine the structure of the novel RDF new approaches of *de novo* model building were used in combination with artificial intelligence (AI). The overall architecture of the 30S-30S dimer shows a head to body orientation. The RDF links the head and body regions of two distinct 30S molecules in the form of a dimer. The binding position of the RDF monomeric structure on the head of 30S subunit implies a mechanistic role to regulate the mRNA binding on the 30S subunit. In turn, the 30S subunits are unable to initiate the translation and stay in a hibernation mode.

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Keywords: ribosome, dimerization factor, cryo-EM, structure

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ON THE INTERFACE BETWEEN MUSCLE WASTING AND CENTRAL NEURODEGENERATION

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Abstract: Compared with Duchenne muscular dystrophy, Miyoshi muscular dystrophy (MMD) belongs to the rare and clinically milder forms. The disease is caused by mutations in the gene encoding dysferlin, a protein involved in the repair of the sarcoplasmic membrane after mechanical stress. In addition to skeletal muscle cells, dysferlin is also abundantly expressed in cardiomyocytes and the brain. Until now, muscular dystrophies are seen as strictly muscular diseases, although it is known that patients with Duchenne muscular dystrophy suffer from a wide spectrum of neurological disorders in the advanced stages of the disease. From the point of view of other types of muscular dystrophy, the involvement and extent of CNS damage in the overall picture of the affected patient is a poorly studied area. In our work, we identified a family with MMD from the Orava region with seven children, 4 of whom are affected by this disease. Genetically, they are compound heterozygotes, where one pathogenic allele is known and the other is newly identified. The main goal of the presented work was to examine the impact of pathogenic mutations in dysferlin on the structural and functional characteristics of the brain and

to identify possible neurological risks in MMD patients, that have been overlooked due to the rarity of this disease, or have not been associated with MMD.

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CELL RESPONSES UPON THYMIDYLATE SYNTHASE INHIBITORY TREATMENTS DIFFER IN MULTIPLE MOLECULAR AND PHENOTYPIC ASPECTS

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Abstract: Besides emerging novel cancer treatment strategies, the traditional chemotherapies are still prevalent in the clinical practice, and they frequently target the thymidylate biosynthesis pathway, particularly the enzyme thymidylate synthase (TS) (PMID: 12819937). The efficiency of a drug treatment depends on the cytostatic effect and the selectivity for cancer cells, but it is also crucial how much the side effects, additional mutagenesis events and the frequently emerging drug resistance can be avoided. All of these aspects depend on the actual drug molecules, as well as on the genomic variations of the cancer cells including defects in DNA damage response and DNA repair genes. Here, we demonstrate multiple alterations between two TS inhibitory drugs applied in derivatives of HCT116 colon cancer cell line with altered DNA repair capacities. We investigated raltitrexed (RTX), the most specific antifolate-type inhibitor of TS, and 5-fluoro-2'-deoxyuridine (5FdUR), the prodrug of the irreversible TS inhibitor, 5-fluoro-2'-deoxyuridine monophosphate (FdUMP). Previously, we have characterized the genomic uracil incorporation induced by these drugs in uracil-DNA repair inhibited cells (PMID: 32956035) using specific uracil-DNA sensors developed in our laboratory (PMID: 26429970). The two drug treatments induced specific and similar genomic uracil patterns correlating mostly with early replication timing and active euchromatin regions (PMID: 32956035). In addition, some drug specific differences were also identified, which now have been further investigated and correlated with alterations in molecular and phenotypic aspects of the cellular responses upon RTX and 5FdUR treatments. Most intriguingly, in DNA repair deficient cells, high dose of 5FdUR leads to temporally decreased cytotoxicity, milder cell cycle arrest and increased frequency of genomic C to T transitions as compared to the low dose 5FdUR or any dose of RTX treatments. Further analysis of the mutational spectra pointed to an APOBEC cytidine deaminase action. We confirmed that upon both drug treatments, several members of APOBEC3 family are induced and localized in the nuclei of these cells potentially attaching the ssDNA portion of the genomic DNA. Besides the cellular regulation of APOBEC enzymes, drug responses reflected in gene expression changes as well as differently perturbed RNA regulatory activity of TS were also addressed. Better

understanding of the molecular context of drug actions on different genetic background can contribute to the repertoire of personalized cancer therapies.

Keywords: thymidylate synthase inhibitors, genomic uracil pattern, induced mutagenesis, APOBEC cytidine deaminase, gene expression

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MODULATING THE PROPERTIES OF RECOMBINANT SPIDER SILK PROTEIN eADF4(C16) HYDROGELS

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Abstract: Hydrogels derived from recombinant spider silk protein eADF4(C16) have gained significant attention due to their unique properties and potential applications in tissue engineering and biofabrication. The influence of sulphate and phosphate salts at different temperatures on fibrillization and the properties of the resulting recombinant spider silk-based hydrogels was investigated. Kinetic measurements at various temperatures showed that the presence of sulphate salts during the self-assembly of recombinant spider silk eADF4(C16) led to the dominant role of secondary nucleation, resulting in increased branching of the formed fibrils. Rheological measurements demonstrated that the addition of salts, which led to different fibril branching, significantly impacted the hydrogels' elastic modulus.

Keywords: recombinant spider silk protein, hydrogels, self-assembly, secondary nucleation, anions

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IDENTIFYING MICROSATELLITE INSTABILITY THROUGH ANALYSIS OF SEQUENCING DATA

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Abstract: Microsatellite instability (MSI) is a genetic condition characterised by higher rate of insertions and deletions in repetitive genomic regions, also known as microsatellites. MSI is caused by impaired DNA mismatch repair (MMR) mechanism responsible for reparation of sporadic insertions, deletions and substitutions introduced during DNA replication. It is associated with colorectal, gastric, endometrial, ovarian, skin, and brain cancers, with the highest prevalence in colon cancers. MSI is also the hallmark of Lynch syndrome (LS), a hereditary genetic condition associated with an increased risk of various cancer development. The majority of LS-associated tumours (90%) exhibit MSI, with colorectal cancers (CRCs) being the most prevalent. In our investigation, whole-genome sequencing data from paired controls and LS-associated CRC samples were evaluated. In order to find unstable microsatellites, we first evaluated STR loci using the MSIsensor tool, whose results were used for subsequent analysis. Hierarchical clustering was utilised to determine the similarity between samples and up-set plot was used to identify MSI loci shared among samples. Out of the 286 030 analysed MSI loci, 270 were present in all samples. Then, we filtered genomic sites in highly repetitive ribosomal DNA regions, which resulted in the appearance of recurrent MSI loci likely unrelated to the LS. The remaining 199 loci may serve as markers for the assessment of LS-associated tumours. The technique demonstrated promise for the detection of oncological disorders and made it possible to detect certain forms of cancer.

Keywords: Bioinformatics, Cancer, Microsatellite Instability, Lynch Syndrome, Whole Genome Sequencing

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NON-OXIDATIVE PHOSPHORYLATION ENERGY-HARNESSING PATHWAYS IN RESPIRATION-IMPAIRED MITOCHONDRIA

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Abstract: Low oxygen tension, mtDNA mutations or other bioenergetic impairments due to pharmacological or genetic interventions lead to severe decreases in oxidative phosphorylation (OXPHOS). Under such conditions, metabolic rewiring may rescue respiration-impaired mitochondria and as an extension of this, the harboring cell. Such metabolic adaptations are frequently observed in solid tumor microenvironments and also animals exceling in withstanding prolonged periods of hypoxia; furthermore, ways of harnessing energy during OXPHOS deficiency would be key in thwarting damage caused by tissue ischemia. Despite the complexity, non-OXPHOS related bioenergetics boil down to four main principles: i) alleviation of 'reductive stress' reflected in the NADH/NAD+ ratio, ii) feeding of the 'Q junction', iii) support of mitochondrial substrate-level phosphorylation (mtSLP), and iv) keeping H2S oxidation in check by modulating Sulfide Quinone Oxidoreductase (SQOR) activity. During the presentation the aim will be to correlate the above principles with specific OXPHOS alterations.

Keywords: Q junction, bioenergetics, sulfide, NADH, mtSLP

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ISOCITRATE DEHYDROGENASES OF TRYPANOSOMATIDS

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Abstract: Isocitrate dehydrogenase (IDH) is an enzyme that catalyses the oxidative decarboxylation of isocitrate to α-ketoglutarate. Depending on its subcellular localisation it can use two electron acceptors – either NAD+ or NADP+. In eukaryotes, IDH1 (NADP+-dependent) can be found in the cytosol and/or peroxisomes. Then there are also two mitochondrial IDHs – IDH2 (NADP+-dependent) and IDH3 (NAD+-dependent). The presence of NADP-dependent IDHs in prokaryotes and lower eukaryotes is thought to be a result of adaptation to highly oxidised compounds available for growth that are energy-poor nutrients. In this case, the pentose-phosphate pathway does not function for production of NADPH and alternative sources of this compound are necessary for biosynthetic processes. NADP-dependent IDHs are one of them. The specificity for cofactor was shown to be dependent only on the five amino acids in the active site. The only NAD+ dependent IDH3 is the enzyme operating in Krebs cycle.

Trypanosomatids are specific when it comes to the Krebs cycle (TCA) because it does not function in its usual cyclic way. The best studied example so far is *T. brucei*. All the necessary eight enzymes are present and their expression was confirmed by proteomic and SILAC data. One of the possible explanations could be low activity of some of the enzymes resulting in the diversion of metabolites by the more active enzymes. The two best candidates for this assumption are aconitase and isocitrate dehydrogenase. Possible usage of NADP by mitochondrial IDH is another hint supporting the hypothesis. The only experimentally characterised IDH of *T. brucei* so far is putatively peroxisomal IDH1 that should be NADP dependent. When purified, this enzyme showed in vitro similar specific activities with both NADP and NAD. In another model trypanosomatid, *Leishmania mexicana*, two isoenzymes are present – NADP-dependent mitochondrial and NAD-dependent without targeting signal, most related to TCA enzymes. Only localisation studies were performed so far.

In our study we would like to shed light onto evolutionary origin and distribution of IDHs in trypanosomatids. We would also like to characterise the preference for cofactor in both mitochondrial and cytoplasmic/glycosomal IDHs. As our models we will use not only well-known and studied models such as *T. brucei* or *L. mexicana*, but also so far understudied monoxenous trypanosomatids and cover the whole phylogenetic tree of these unicellular parasitic organisms.

Keywords: trypanosomatids, mitochondria, isocitrate dehydrogenase

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PROLINE SEMI-SPECIFIC ENZYMES IN THE SPOTLIGHT: THE ROLE OF *An*PEP IN STRUCTURAL PROTEOMICS

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Abstract: Proteolysis plays a crucial role in structural proteomic workflows such as hydrogen/deuterium exchange (HDX) where it provides a spatial resolution and cross-linking (XL) or fast photochemical oxidation of proteins (FPOP) where it enables precise localization of modifications. However, some proteins appear to be challenging analytical targets not easily amenable by commonly used proteases. Thus, the search for new cleavage tools is of high importance. Recently, the utility of proline semi-specific enzymes such as *Aspergillus niger* prolylendopeptidase (*An*PEP, ProAlanase) has been highlighted.
In this study, we used the industrial grade *An*PEP and tested it under conditions described for research grade ProAlanase leading to (semi)specific digestion. In addition, we immobilized *An*PEP and tested prepared columns under specific (extremely low pH) and HDX-compatible (pH 2.5) conditions. For HDX-MS, we used *An*PEP columns alone and in combination with other non-specific proteases. This we demonstrated on clinically relevant prion proteins where the implementation of *An*PEP is the key due to Ala and Pro rich sequences. Under the specific conditions we discovered previously overlooked cleavage dependencies and showed that the industrial grade *An*PEP is a good source of enzyme available in quantities suitable for immobilization and generation of protease columns.
We also developed new java-based software tool DigDig facilitating fast and easy comparison and parametrization of protein digestions, extraction of cleavage preferences and working well even with complex mixtures.

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Keywords: prolylendopeptidase, Aspergillus niger, proteolysis, structural proteomics, mass spectrometry

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INTERPLAY BETWEEN P-GLYCOPROTEIN MEDIATED DRUG RESISTANCE AND THE LPHN1/GAL-9/TIM-3 SIGNALING PATHWAY

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Abstract: P-glycoprotein (known as ABCB1 transporter) expression in myeloid blasts of acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) leads to the commonly observed multidrug resistance. Overexpression of latrophilin-1 was detected in leukemic cells from AML patients. We showed that ABCB1 overexpression is associated with decreased latrophilin-1 expression in MOLM-13/VCR and SKM-1/VCR AML cell variants derived from MOLM-13 and SKM-1 cells by vincristine selection/adaptation. We also found that if ABCB1 overexpression occurs in myeloid blasts of newly diagnosed MDS patients, latrophilin-1 expression is attenuated. Latrophilin-1 may initiate TIM-3- and galectin-9-mediated immune escape. We demonstrated changes in the expression of both proteins by comparing ABCB1-positive cell variants (MOLM-13/VCR, SKM-1/VCR) with their ABCB1-negative counterparts. Galectin-9 was present in our cell lines in eight protein isoforms for which we identified the respective transcription variants resulting from alternative splicing, and we verified their structure by sequencing. The isoform profile of galectin-9 was different between ABCB1-positive and ABCB1-negative cell variants. The interaction partner of galectin-9 is CD44, and its expression was altered in the ABCB1-positive variants MOLM-13/VCR and SKM-1/VCR compared to their ABCB1-negative counterparts.

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Keywords: ABCB1; latrophilin-1; CD44; TIM-3; galectin-9

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VIBRIO NATRIEGENS: A PROMISING EXPRESSION HOST FOR THE PRODUCTION OF "DIFFICULT-TO-EXPRESS" RECOMBINANT PROTEINS

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Abstract: When aiming for industrial production, it is preferable to utilize fast-growing, well-known, and cost-effective bacterial hosts. Novel prokaryotic platform based on *V. natriegens* offers many advantages over widely used host systems for protein production at laboratory and industrial scales. *V. natriegens* is gram-negative, non-pathogenic bacterium with shorter doubling time than *E. coli* (< 10 min). It has a rapid proteosynthesis and exceptionally high biomass specific consumption rate (qs) under aerobic and anaerobic conditions. Moreover, *V. natriegens* possesses a robust metabolism, utilise various substrates and grows rapidly on low-cost media. We have used this host platform for intracellular production of various model proteins such as mutated MMLV reverse transcriptase (RT). We were able to gain significantly higher soluble fraction of mutated MMLV RT comparing to standard *E. coli* BL21(DE3) strain. That suggests the versatility of this host platform and provides an alternative to *E. coli*. We also introduced an alternative strategy for production of recombinant proteins directly into growth medium using co-expression of D,D-carboxypeptidase enzyme PBP5/6 in *V. natriegens*. PBP5/6 is low molecule penicillin binding protein associated with cell membrane which plays an important role in peptidoglycan metabolism. The imbalance in levels of this enzyme could contribute to outer structure instability and leakage of intracellular proteins into growth medium. We used model recombinant proteins such as AfKatG or mangan-dependent peroxidase DypB for demonstration of the leakage into growth medium. For instance, after purification from constant volume of growth medium using affinity chromatography (IMAC) (at the same conditions) we were able to reach yields of 117.9 (± 56.0) mg/L when PBP5/6 co-expressed. In control cultivation, the total gained yield of AfKatG purified from growth medium was 3.2 (± 1.3). Total purified yield of MDBP from medium using one-step IMAC was ± 36.5 mg/L resulting in increase compared to the control. This production strategy not only has the potential to accelerate downstream processes and reduce overall production costs but also serves as a promising alternative for the production "difficult-to-express" recombinant proteins with low solubility in *V. natriegens* strains.

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Keywords: recombinant proteins, *Vibrio*, expression

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PRODUCTION OF RECOMBINANT LECTIN PluLec FROM *Photorhabdus luminescens* FOR NEUTRON CRYSTALLOGRAPHY AND ITS STRUCTURE-FUNCTIONAL CHARACTERIZATION.

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Abstract: Lectins are ubiquitous proteins of non-immune origin that reversibly and specifically interact with carbohydrates. They play a role in various physiological and pathological processes like intercellular communication, adhesion, migration and host-pathogen interactions. Unlike antibodies, they are not products of immune response and lack enzymatic activity. Lectins are widely used for carbohydrate structure characterization, glycoprotein purification, and specific labeling of cell surface structures.
Photorhabdus luminescens is a bioluminescent Gram-negative bacterium and an insect pathogen that symbiotically lives in Heterorhabditidae nematodes. PluLec, a putative lectin from *Photorhabdus luminescens* shares homology with PA-IL lectin, which is D-galactose specific, Ca^{2+} dependent, cytotoxic lectin from opportunistic pathogen *Pseudomonas aeruginosa*, involved in facilitating infection in patients with compromised immunity.
This research is focused on structural-functional characterization of recombinant protein PluLec using various methods like isothermal titration calorimetry, hemagglutination, glycan array, analytical ultracentrifugation, toxicity tests performed on insect models and protein X-ray and Neutron crystallography. Unraveling lectin's atomic structure and interactions with carbohydrates is essential for understanding its function and potential applications. Neutron protein crystallography, with its unique ability to visualize hydrogen atom positions, offers a promising avenue for gaining deeper insights into lectin-carbohydrate recognition.
However, the successful application of neutron crystallography requires producing sufficient quantities of isotopically labeled recombinant lectin and obtaining high-quality crystals suitable for neutron diffraction. Several considerations, such as selecting an appropriate expression system, optimizing expression and crystallization conditions, need to be taken into account.

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The study revealed that lectin crystallizes as a homotetramer with four binding sites for D-galactose (one per monomer) and shows specificity towards beta anomers of D-galactose. Preliminary results from toxicity tests conducted on insect models indicate a clear negative effect on insect survival. The obtained results of the structure and function of PluLec may reveal its importance in the pathogenic or symbiotic stage of life. Neutron diffraction experiments can deepen our understanding of lectin functionality, aid in the design of therapeutic strategies targeting lectins, and potentially inspire the development of novel carbohydrate-based therapeutics.

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Keywords: lectin, structure-functional analysis,

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WHY SHOULD WE CARE ABOUT GENDER EQUALITY IN SCIENCE?

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The contribution introduces key concepts and problems related to the topic of gender inequality in the field of science. It provides an insight into the situation of women in science in Slovakia and in the EU with specific focus on the field of biochemistry. We will talk about the phenomenon of glass ceiling and leaky pipeline and discuss the role of working conditions and safe institutional environment in promoting careers of women in research. We will also touch upon the integration of gender dimension in research and its importance for increasing the quality and relevance of research - including in biochemistry.

Keywords: *Gender equality, Gender dimension in research, Careers in science*

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POST-GRADUATE STUDY PROGRAM IN BIOCHEMISTRY AT THE FACULTY OF SCIENCES OF PAVOL JOZEF ŠAFÁRIK UNIVERSITY IN KOŠICE

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Abstract: The Department of Biochemistry at the Faculty of Sciences of the Pavol Jozef Šafárik University in Košice provides teaching at the bachelor's, master's and doctoral levels based on a credit system of study. The Department offers a post-graduate study program in the field of the structure and function of biomacromolecules. A substantial part of the content of the doctoral study program is devoted to familiarising students with the latest scientific findings in biochemistry, molecular biology, biophysical chemistry, bioinformatics and the conformational stability of biomacromolecules. Graduates of the Biochemistry doctoral study program are given the opportunity to work independently and demonstrate a creative scientific approach. They gain a systematic, comprehensive understanding of the fields of biochemistry, molecular chemistry and clinical biochemistry, including an appreciation of the interrelationships between these fields and with other related disciplines. They have mastered scientific research methodologies with a focus on using progressive research techniques in biochemistry at a level that meets international criteria. Graduates are capable of identifying promising new directions of research, formulating scientific hypotheses, compiling research projects, selecting appropriate experimental techniques to examine specific topics, and leading research teams. Graduates of the doctoral program can go on to develop their careers in research institutions as well as in educational institutions in Slovakia and abroad, environments in which they can creatively improve and develop their scientific theories, contribute to research and development, and implement innovation procedures. Graduates are also able to assist in teaching specialized chemistry subjects at universities and to conduct research independently. The theoretical and practical knowledge acquired in the program enables graduates to manage teams of technical staff, teach graduates of master's and bachelor's studies in chemistry and take responsibility for identifying and implementing comprehensive and complex solutions.

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Keywords: Biochemistry, Doctoral degree

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TEACHING OF BIOCHEMISTRY AT FCHPT STU - IMPACT OF NEW ACCREDITATION, COVID-19 AND THE PROJECT "ACCORD"

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Abstract: Teaching of biochemistry at the Faculty of Chemical and Food Technology STU in Bratislava has a long tradition. Over the more than 70 years, the teaching of biochemistry and related subjects has expanded from originally one study programme to the current five study programmes in the course of bachelor studies, and three study programmes in the course of engineering studies. The scope of biochemistry teaching is not the same in all programmes with regard to the needs and orientation of students. And, of course, the teaching of biochemistry also continues during the PhD programme, but here more in the form of individual consultations and self-study. However, during the whole period of teaching biochemistry at our faculty there have not been such significant restrictions as in the last three years, during which we were affected by the Covid-19 pandemic, the reconstruction of one of the faculty buildings and the transition to the new system of accreditation and evaluation of the quality of studies in higher education institutions.
The aim of the presentation will be to provide more information about organisation of teaching biochemistry, position of biochemistry in curricula, methods of teaching, continuity of study subjects and several other aspects of teaching biochemistry at our faculty.

Keywords: teaching, biochemistry

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INFECTION SITE MOLECULAR MAPPING

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Abstract: The onset of invasive infection in the inflammation site encompasses many molecules with diverse molecular sizing and chemical properties. Some of these molecules are highly ephemeral, with prompt turnover rates. By mass spectrometry and microscopy we semi-quantified selected molecular events during bacterial and fungal evasion in two rat infection models (**i** and **ii**). Host response was evaluated by monitoring the mammalian proteinaceous, peptide, and siderophore biomarkers spread from the inflammation site to distant bodily fluids. The concentration levels of microbial siderophores secreted by *Escherichia coli* and *Rhizopus microsporus* during their proliferation represented the quantitative biomarkers of viable pathogens. **(i)** Bacterial neuroinfection was induced by intracranial injection of a 5×10^6 CFU *E. coli* 5172 suspension into the brains of immunocompetent female Lewis rats. Recorded in the cerebrospinal fluid (CSF), we demonstrated the severity of the infection by the exponential increase of microbial salmochelins SX, S1, S2, and S5; 2,3-dihydroxybenzoylserine, and aerobactin copy numbers reflecting the bacterial log phase. Salmochelins, glycosylated enterobactin analogs, cannot be trapped by lipocalin-2 due to their bulkier structures and evade the immune system while preserving their iron-chelating properties. Non-glycosylated enterobactin was less abundant in the CSF. Host secretoneurin concentrations doubled in the CSF during the 20 hours of infection development. The distribution of deprotonated salmochelin SX and aerobactin molecules in tissue reflected the infection sites and correlated with electron microscopy images. The SEM on consecutive sections provided biofilm-like structures with extracellular matrix formation. **(ii)** Fungal pulmonary infection was induced by intratracheal application of 1×10^8 *R. microsporus* CFUs administered in 100 µL volume. Cyclophosphamide (i.p., 75 mg/kg) was applied 5 and 1 days before the infection to induce neutropenia. In 3/5 of animals, the massive neutrophil infiltration into the inflammation sites documented through defensins detection the re-established immune response. Lower fungal siderophore concentration levels were detected in the lung tissue and animal urine. In microscopy images, we saw clearance of spores and hyphae that correlated with the decline of rhizoferrin and homorhizoferrin carboxylates in a five-day experiment. In the sickest animal, rhizoferrin urine concentration reached 306.8 µg/mL four days post-infection. The lung parenchyma collapsed and were intercalated with hyphae and spores. **Conclusion:** We can monitor the molecular interplay at the host-pathogen interface through siderophore secretion kinetics (tissue and bodily fluids) and semi-quantitation of host immunomodulatory peptides and proteins at the infection site. Precise host-pathogen interactome quantitation can provide outcome prediction in an infected host.
Acknowledgement: Czech Science Foundation 21-17044S and 22-06771S.

Keywords: microbial infection, siderophore, mass spectrometry, microscopy

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NOVEL ASPECTS OF BENZO[A]PYRENE TOXICITY

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Abstract: Benzo[a]pyrene (BaP) belongs to the most important environmental and occupational carcinogens that has been primarily studied as mutagenic and genotoxic compound. Its mutagenicity/genotoxicity largely depends on aryl hydrocarbon receptor (AhR)-mediated oxidative metabolism leading to the production of dihydrodiol epoxide metabolites, as well as additional metabolites contributing to oxidative DNA damage. The AhR is a ligand-activated transcription factor, which plays a major role in toxic effects of environmental pollutants. It is a pivotal regulator of several xenobiotic-metabolizing enzymes (XMEs), and is now considered to play an important role also in control of cell cycle, apoptosis and cell differentiation. In the present review, some of the known non-genotoxic effects of BaP-AhR axis are summarized, including various effects on cell membrane, activation of intracellular signaling pathways, crosstalk between AhR and other transcription factors and disruption of lipid and glucose metabolism.
The accumulating evidence suggests that there exists a multiple crosstalk between AhR activation by BaP and the signaling pathways activated by inflammatory mediators, such as nuclear factor-kB, a pleiotropic transcription factor controlling the immune/inflammatory responses. These include altered expression of proinflammatory cytokines, such as tumor necrosis factor-alpha or interleukin-6, and deregulation of expression/activity of principle enzymes producing inflammatory mediators, such as cyclooxygenase-2. A number of studies have also indicated that BaP may, either directly or indirectly (via AhR activation), interfere with nuclear steroid receptors, in particular, with estrogen and androgen signaling, leading to endocrine disruption. Several recent papers paper bring novel data on effects of BaP during gradual process of epithelial-mesenchymal transition (EMT), an essential mechanism for metastatic dissemination, which is associated with enhanced cell migration, invasion, and altered cell morphology. Thus, BaP can contribute also to another key step in cancer development. Taken together, BaP exposure elicits multiple modes of action leading to disruption of endogenous energy metabolism, endocrine signaling, pro-inflammatory and carcinogenic processes.

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MOLECULAR GENETIC CHANGES IN LYMPHOID MALIGNANCIES AND THEIR IMPACT ON THE PATHOGENESIS, PROGNOSIS AND MOLECULAR EVOLUTION OF THE DISEASE

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Abstract: Lymphoid malignancies are considered as incurable diseases due to the limited ability of current treatment options to completely eradicate leukemic or lymphoma cells. Genetic changes present in tumor cells represent the key factors affecting the pathogenesis and prognosis of the disease and influence the response both to chemoimmunotherapy and novel treatment options. The therapy selection pressure frequently favors the preferential proliferation of rare resistant cells, leading to recurrent relapses. Chronic lymphocytic leukemia (CLL) as the most frequent adult leukemia manifests remarkable clinical variability linked with extensive heterogeneity of genomic defects. Despite the progress with novel therapeutic agents, some patients still face disease persistence due to subclonal populations, clonal evolution of genomic defects is observed mainly in treated patients. The presence of genetic aberrations in antioncogene *TP53* is the key prognostic and predictive factor in lymphoid malignancies, especially in chronic lymphocytic leukemia and some lymphomas. The *TP53* gene mutations could be present even in minor clones of CLL cells, which results in the significantly worse prognosis of the patients and need of the modern biological therapy based on the BCR or Bcl-2 inhibition (Malčíková et al., Blood 2021). TP53 gene belongs among the most frequently mutated genes in anaplastic large cell lymphoma (ALCL), together with STAT3 mutations, that are associated with shorter patient´s survival (Lobello et al., Leukemia 2021). Activity of p53 protein is influenced not only by gene aberrations, but epigenetic changes also play a role in the protein behavior. We have analysed also the impact of post-translational modifications such as phosphorylation (Mančíková et al., Mol. Oncol. 2023) or methylation of prognostically relevant gene promoters (Poppová at al., Epigenetics 2022).
The diagnostic and clinical need for a detailed and sensitive analysis of prognostic and predictive genes resulted in the development of diagnostic gene panel called LYNX (LYmphoid NGS) that is a very useful tool for biomarker detection, including complex immunoglobulin genes and chromosomal translocations (Navrkalová et al., J. Mol. Diag. 2021). The use of such diagnostic

gene panel is essentially coupled with special bioinformatic solutions of NGS data (Hynšt et al., Peer J. 2021, Tauš et al., Front. Gen. 2021). The identification of relevant prognostic markers, analysis of their biological role and implementation of novel diagnostic techniques into the medical practice need a variety of complex approaches, that could bring novel findings on the disease pathogenesis as well as new ways of patient diagnostics.

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Keywords: leukemia, genetic aberrations, TP53 gene, clonal evolution

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HOW IS CHANGING ROLE OF CHEMICAL SUBJECTS IN THE MEDICAL STUDY CURRICULUM LAST DECADE?

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Abstract: The formation of chemistry dealing with processes in living organisms took place in medical schools, so it is not surprising that medical chemistry was one of the first designations taught at the beginning of the 20th century. Gradually, knowledge about metabolism, enzymes, hormones, and vitamins increased, and it started to use the name physiological chemistry; later, the name biochemistry was used more and more. The enormous increase in knowledge about new molecules, nucleic acids, and signaling brought further splitting and the emergence of new branches. In many medical faculties, are subjects such as, e.g. molecular biology and pathobiochemistry taught separately due to the huge increase in knowledge in this area and the importance that the knowledge of biochemical processes at the molecular level is gaining. However, the present day is characterized too by an interdisciplinary approach to the study, which makes it possible to look at the studied problem from different angles and thus enables a better understanding of the mechanisms. However, it is necessary to realize that with such a massive increase in knowledge, it is challenging to choose the most important things that will be necessary for future doctors not only during their studies and immediately after but will also prepare them for the knowledge that is yet to come, or that are waiting to be discovered. Today's trend is also the connection with practice and the best possible preparation for real life.

At the beginning of the 21st century, the practical use of acquired knowledge in clinical practice is also coming to the fore in Slovakia, leading to the implementation of clinical biochemistry in teaching future doctors. Clinical biochemistry integrates knowledge from clinical and preclinical subjects (e.g., medical biochemistry, physiology, pathophysiology), and students are aware of its importance, evidenced by their request to expand the teaching hours. Clinical-biochemical laboratory examinations and methods form a significant part of medical procedures associated with preventive measures, diagnosis of diseases, or monitoring of treatment. Knowledge of the current possibilities or limitations of the use of laboratory tests is an excellent aid in the daily practice of future doctors.

The trend we observe in the gradual separation of medical chemistry, biochemistry, medical biochemistry, or clinical biochemistry from the field of chemistry is also visible in the specializations of people teaching these chemical subjects at medical faculties. The amount of knowledge, on the one hand, and the narrow specialization, even the atomization of the fields, prevent a more comprehensive approach to studying the mechanisms in the human body at the molecular level.

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Keywords: chemistry, medical chemistry, biochemistry, clinical biochemistry

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PRODUCTION AND CRYSTALLIZATION OF LECTIN FROM *PHOTORHABDUS LAUMONDII*

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Abstract: Lectins are ubiquitous proteins with the ability to reversibly bind to the mono-, oligo- and polysaccharides with high specificity. These sugar-binding proteins can be found in most organisms, ranging from viruses and bacteria to plants and animals. They play an essential role in many biological processes, such as cell-cell interaction or recognition of the host by a pathogen. Lectins represent a heterogeneous group of proteins that vary in size, oligomeric state, and structure. Due to their importance, lectins are studied structurally and functionally to thoroughly understand their role and mechanism of action [1]. Research is conducted on the lectins from gram-negative entomopathogenic bacteria *Photorhabdus asymbiotica*, which live in symbiosis with *Heterorhabditis* nematodes. This symbiotic complex can be found in soil, where it searches for insect prey [2]. Besides functional characterisation, structural information is essential for discovering the number of binding sites, the key residues involved in the interaction, and the binding partner's orientation. For this purpose, protein crystallography was used to determine the 3D structure of lectin PLU1 and its complex with binding partners in atomic resolution. One of the most critical factors for protein crystallisation is protein sufficient purity. Purification of PLU1 required unique approaches to reach purity for its crystallisation. Examination of the PLU1 structure revealed a unique binding pocket, which significantly impacts the binding properties of the PLU1.

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Keywords: Photorhabdus, laumondii, lectin, purification, crystallization

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HOW DO AQUATIC PLANTS COPE WITH PATHOGENS IN CHERNOBYL ENVIRONMENT?

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Abstract: Plants face unclear fitness consequences due to prolonged exposure to low doses of ionizing radiation. Radiolysis of macromolecules generates genotoxic free radicals. They could trigger multiple signalling cascades in plants, and modify their morphology and physiology. As a defence response, plants frequently synthesize specific antioxidants, proteins, and metabolites. We hypothesized that plant resistance to simultaneous biotic stress could be compromised. In essence, our study focused on revealing biochemical mechanisms responsible for the reaction of chronically irradiated wild aquatic plants (common reed—*Phragmites australis*) to successive biotic stress. The mature leaves and seeds were collected from the lakes in Chernobyl Zone contaminated with radionuclides ^{137}Cs and ^{90}Sr and clean lakes in neighbouring areas. The difference in the plant resistance was observed through leaf-sheath assay. It confirmed that leaves from contaminated lakes were more susceptible to fungal infection than those from clean lakes. In an ongoing experiment, we are testing fungal resistance in the next generation of plants grown in controlled laboratory conditions. To decipher the mechanism, protein profiling of collected leaves was performed using ultrahigh-performance liquid chromatography and mass spectrometry. We quantified 1340 proteins, and among them, 174 proteins were differentially accumulated between the two control and contaminated locations. However, principal component analysis indicated that sampling variables had a higher impact on proteome than contamination with radionuclides. Moreover, the level of antioxidants was not affected in reed leaves exposed to chronic ionizing radiation. Nevertheless, we observed higher DNA damage in the seedlings from the contaminated lakes. Since protein carbonylation is an irreversible marker of oxidative damage, quantifying the changes in site-specific carbonyls using affinity enrichment and mass spectrometry is the further goal of our study. The outcomes of our research could bridge fundamental radiobiology and relevant management practices for contaminated lakes.

This study was supported by the projects APVV-20-0545 and VEGA 2/0106/22.

Keywords: ionizing radiation, *Phragmites australis*, protein carbonylation, fungal resistance

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APPLICATION OF NANOTECHNOLOGY-BASED PHOTONICS SENSORS IN ENVIRONMENT, MEDICINE, AND FOOD CONTROL

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Today, we are witnessing an increased interest in the market demand for high-quality, low-cost, and safe sensing devices. It is caused by the fact that traditional methods applied in may take days to weeks to obtain results and often require investment in capital costs as well as time for sample preparation. Therefore, we observe a large increase in applications of various types of sensors, including a significant increase of micro- & nano-sensors. Micro- & nano-sensors provide real-time monitoring compared to traditional detection methods such as chromatography, mass spectrometry and different spectroscopic methods.

PickMol™ technology is based on plasmon enhanced Raman scattering. This technology detects sub-nanomolar concentration of different organic molecules in various matrixes (water, food soil, air). PickMol™ carrying out an *in-situ* analysis with the same sensitivity (ppb level) as reached by certified methods (i.e. GC-MS). The patented PickMol™ technology was validated by certified laboratory and can be tailored for any organic molecule, which means its large potential for application in wide range of fields like pharmaceutical & chemical industry (cleanliness), security (explosives, toxins), sport (doping), environment (pollutants), medicine (viruses, onco-markers…), foods (beverages…).

PickMol™ technology is:

- ✓ Sensitive (ppb concentration level)
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Application of PickMol™ technology for the detection of pollutants, micro- & nano-plastics, viruses, and volatile organic compounds (VOCs) will be presented.

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CLINICAL APPLICATION OF CELL-FREE NUCLEIC ACIDS

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Abstract: Clinical application of cell-free nucleic acids (cf-NAs) became a promising diagnostic possibility. Samples are collected by the non-invasive liquid biopsy for this utilization. Cell-free DNA isolated from the maternal blood is widely used in prenatal testing to detect fetal genetic disorders. Beside that, analysis of cf-DNA may provide information about the mutation profile of tumor cells, while cell-free non-coding RNAs are promising biomarker candidates in the diagnosis and prognosis of cancer. These biomarkers have the potential to help clinicians in therapy selection and in the follow-up of patients. Thus, cf-NA-based diagnostics represent a new possibility in personalized and preventive medicine. They will have a tremendous effect in future screening, diagnosis, prognosis, follow-up and treatment of cardiovascular diseases, cancer, diabetes and other diseases. Currently, the use of circulating cf-DNA and cf-RNA markers is investigated mostly in cancer prediction and screening, monitoring treatment and recurrence, detection of resistance to therapy as well as minimal residual disease. Despite their great promise in cancer diagnostics, only a few cf-NA-based liquid biopsy tests have been approved for clinical use. Still, despite all obstacles and limitations, the future is to develop of molecular diagnostics and bioinformatics into account, the application of liquid biopsy based multivariate diagnostic tests. These tests will open a new path in personalizedand preventive medicine and healthcare in the future.

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Szilágyi M, et.al.. Circulating Cell-Free Nucleic Acids: Main Characteristics and Clinical Application. Int J Mol Sci. 2020 Sep 17;21(18):6827

Keywords: cell-free nucleic acids, liquid biopsy, biomarker, oncology,

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AUTOPHAGY AND APOPTOSIS SWITCHING DURING PHOTO-ACTIVATION OF CANCER CELLS

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Abstract: Nowadays, photoactivation in combination with other therapeutic approaches is an attractive adjuvant modality for the treatment of cancer. Targeted destruction of cancer cells and stimulation of healthy cells is one of the main advantages of photoactivation. In the present work, we have shown that the combination of irradiation with two wavelengths (at 808 nm and 590 nm) can lead to different responses in cells treated with hypericin, depending on their origin. The study presented here shows significant differences between glioblastoma cells and non-cancerous human skin fibroblasts. This work focuses on mitochondria and an interplay between mitochondrial and autophagic proteins that play a critical role in cell response to photoactivation. Fluorescence microscopy, flow cytometry, and Western blot analysis were used to examine the autophagic profile of the cells after treatment. Overall, we found that the switching of autophagy and apoptosis was dose-dependent. While 590 nm light induced phototoxic changes in hypericin-treated cells, 808 nm light stimulated autophagy rather than apoptosis. Moreover, low concentrations of hypericin in human fibroblasts under 808 nm light irradiation did not cause lethal damage. On the contrary, fibroblasts were forced to overcome the phototoxic effects that were lethal to glioblastoma (under 808- and 590-nm light irradiation). Thus, the combination therapy seems to be promising not only for cancer treatment but also for reducing the side effects of the treatment.

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Keywords: photodynamic therapy, photobiomodulation, apoptosis, autophagy, mitochondria,

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EXPLORING THE PROTEOME AND THE PHOSPHOPROTEOME USING ION MOBILITY SPECTROMETRY

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Abstract: During the LC-MS analysis of complex protein samples, complexity reduction is of paramount importance using various techniques, of which ion mobility spectrometry (IMS) is becoming increasingly popular. A major advantage is that the sample is fractionated on-line in the gas phase, so that no off-line fractionation (e.g. high-pH reversed-phase fractionation) is required during sample preparation. One type of IMS technique is high-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS). Using high- and low-electric fields supplemented with a low DC voltage, the FAIMS interface allows only selected ion populations to pass from the ion source to the mass spectrometer.
In our study, LC-MS measurements were performed with and without FAIMS to investigate if FAIMS can increase the proteome and phosphoproteome coverage of tissue and body fluid samples. Furthermore, we compared quantification results of TMT-labeled samples using MS2-based quantification in combination with FAIMS or the conventional SPS-MS3 method without FAIMS.

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Keywords: ion mobility spectrometry, FAIMS, phosphopeptides

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IDENTIFICATION OF MULTIVARIATE MITOCHONDRIAL FITNESS ALGORITHM IN DIAGNOSTICS OF PARKINSON'S DISEASE

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Abstract: Parkinson's disease (PD) has become the most prevalent neurodegenerative disease. It is a chronic, progressive, treatable, but still incurable dopaminopathy that significantly affects the quality and length of life of the sufferer. While motor symptoms are prevalent, cognitive decline and severe psychosocial issues are frequently linked to the disease's course. Levodopa, dopamine agonists (rotigotine), and MAO-B inhibitors (selegiline, rasagiline) are irreplaceable in PD therapy. DBS therapy is the most effective of the non-pharmacological approaches. The majority of PD cases are sporadic types, whose onset is primarily due to causes other than genetics. The onset of idiopathic PD is usually in the seventh to eighth decade of life, but there are also rare cases with an earlier onset. Similar to other neurodegenerative diseases, PD is characterized by a long prodromal phase that can last several decades. The diagnosis of PD is elaborate and time-consuming. It is supported by a thorough neurological examination, nuclear medicine (DATSCAN, Ioflupane-123I), and a favorable compensatory response to levodopa treatment. Apart from the known PD-related genetic markers, which are not present in the idiopathic form of PD, there are no reliable biochemical or physiological markers in clinical practice. Although the RT-QuIC approach for detecting pathogenic alpha-synuclein currently shows promise, it is not yet ready for routine usage in clinical settings. Therefore, finding accurate disease markers or markers that may be used to forecast the course of a disease and are simple to use in practice is of highest importance. In our laboratory, we are searching for PD-markers among the parameters defining mitochondrial fitness in lekocytes. Recently, using machine learning, we discovered an algorithm that can discriminate PD patients from non-PD controls with about 95% likelihood. Perhaps this finding is a major step towards expediting the diagnostics of PD.

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IRON-SULPHUR CLUSTER ASSEMBLY IN A PROTIST WITHOUT MITOCHONDRION

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Abstract: *Monocercomonoides exilis* is the first eukaryotic organism described as a complete amitochondriate, yet it shares common features with heterotrophic anaerobic/microaerophilic protists, some of which bear divergent mitochondrion-related organelles or MROs. It has been postulated that the retention of these organelles stems from their involvement in the assembly of essential cytosolic and nuclear FeS proteins, whose maturation requires the evolutionarily conserved mitochondrial ISC and cytosolic CIA machineries. The amitochondriate *M. exilis* lacks genes encoding the ISC machinery, yet contains a bacteria-derived SUF system, composed of the cysteine desulfurase SufS fused to SufD and SufU, as well as the scaffold complex SufB-SufC. Here, we show that expression of the M. *exilis SUF* genes, either individually or in tandem, can complement the maturation of the FeS protein IscR in the double mutants of Δ*sufs/iscs* and Δ*sufb/iscua*. *In vivo* and *in vitro* studies indicate that purified *M.exilis* SufB, SufC and SufDSU proteins interact suggesting that they act as a complex in the protist. *M. exilis* SufBC can undergo conformational changes in the presence of ATP and assemble FeS clusters under anaerobic conditions in presence and absence of ATP *in vitro*. Altogether, these results indicate that the dynamic MeSufDSUBC proteins may function as a FeS cluster assembly complex in *M. exilis* thereby being capable of replacing the organelle-enclosed ISC system of canonical eukaryotes.

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PROTEOMIC STORIES/DETERMINING THE PHOSPHORYLATION MAP OF PLANT RETINOBLASTOMA-RELATED (RBR)PROTEIN IN PROLIFERATING TISSUES

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Abstract: Plants, similar to animals, control cell proliferation, cell differentiation and survival by a conserved CYCD-RBR-E2F regulatory pathway. The single RETINOBLASTOMA-RELATED (RBR) in Arabidopsis interacts with many different proteins but E2F transcription factors are believed to be its primary effectors. CDK-CYCD could phosphorylate RBR on as many as 16 putative sites, and the conserved serine 911 site was found phosphorylated and suggested to release E2Fs from RBR repression at the G1 phase resulting in cell cycle entry. We confirmed that phosphorylated RBR at S911 site (P-S911) was never found in complex with E2Fs, but surprisingly other phosphorylated RBR forms at various sites retain its E2F-binding ability suggesting that each phosphorylation might have unique effect on RBR. To investigate whether P-S911 is associated with other phosphorylation events, RBR was immunoprecipitated from young Arabidopsis seedlings by using a phospho-specific RBR antibody recognizing P-S911.
Accordingly, RBR was found to be phosphorylated at nearly all other predicted CDK phospho-sites suggesting that P-S911 might prime phosphorylation at other CDK sites just like in animals. Moreover, neither E2F nor DREAM components were identified in the P-S911 interactome indicating that multi-phosphorylated RBR could not bind to E2Fs and their multi-subunit complexes. Additionally, we confirmed that plant RBR-related proteins purified from young Arabidopsis seedlings, Medicago truncatula root tips and young Brassica napus leaves could be highly phosphorylated at common sites in proliferating cells.

Keywords: proteomics, PTM, cell cycle, signalling, interactions

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THE POTENTIAL OF LIQUID BIOPSY IN THE ERA OF PRECISION MEDICINE

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Abstract: Many serious pathologies, including cancer, manifest with minimal or no obvious phenotypic signs in the initial stages, making early detection and subsequent effective treatment challenging for contemporary medicine. Analysis of cell-free DNA has shown potential in identifying mutations that could represent early neoplastic changes. Here, we present the concept of a liquid biopsy-based test to identify genomic alterations, holding high potential for biomedical applications. We discuss the results of applying powerful genomic technologies and bioinformatic approaches to analyze plasma samples from patients with different malignancies. We emphasize the high diagnostic potential offered by the aggregate evaluation of an extensive set of sequencing metrics and genomic attributes facilitated by a neural network-based machine learning model. Our findings elevate the application of liquid biopsy within the realm of modern personalized medicine, demonstrating its potential as a non-invasive screening tool for multiple cancers.

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Keywords: cancer, screening, liquid biopsy, cell-free DNA, massively parallel sequencing

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TO BE UNDER STRESS, OR NOT TO BE UNDER STRESS, THAT IS THE QUESTION

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Abstract: To maintain health, the cells and tissues of human body have to adapt to the different environmental and pathological conditions, including nutrient and oxygen deprivation, viral infection and protein homeostasis defects. The integrated stress response (ISR) is an evolutionarily conserved intracellular signalling network that helps the cells and tissues to cope with stress conditions and to restore cellular and tissue homeostasis. ISR is regulated by four specific kinases (PERK, GCN2, PKR and HRI) that catalyse phosphorylation of the eukaryotic initiation factor eIF2α, resulting in a general reduction of protein synthesis. Paradoxically, phosphorylation of eIF2α also triggers the translation of specific mRNAs producing key transcription factors, such as ATF4, that drive transcription of genes involved in either restoration of cellular homeostasis or initiation of different forms of cell death. In addition to ISR, cells respond to the non-physiologic conditions or disturbances in cytoplasm, endoplasmic reticulum and mitochondria via specific responses such as proteasomal, ER and mitochondrial stress, respectively.

Proteasomal stress as a response to the overload or dysfunction of ubiquitin proteasomal system (UPS) is characterised by accumulation of aggregates of ubiquitin conjugated proteins and expression of HSP70 that translocates aberrant proteins to lysosomes for their degradation. It can also lead to the activation of HRI kinase (that is part of ISR).

ER stress triggered mainly by disturbances of protein synthesis and processing in ER induces unfolded protein response (UPR) via activation of three ER membrane receptors (PERK that is part of ISR, IRE1 and ATF6). UPR aids to restore homeostasis by translation reduction, induction of the expression of ER-specific chaperones and the activation of ER-associated degradation (ERAD). ERAD includes retro-translocation of misfolded/aberrant proteins from the ER to the cytoplasm, their labelling with ubiquitin and consequent proteasomal degradation.

Mitochondrial stress is a response to different forms of mitochondrial dysfunction including OxPhos inhibition, depolarisation of mitochondrial membrane potential, mitochondrial protein import and processing. It leads to the activation of HRI kinase and UPR specific for mitochondria that depends mainly on ATF5-dependent induction of expression of mtHSP60 and protease LONP1.

Depending on the severity or duration of stress, different forms of cell death may also be initiated resulting in tissue dysfunctions and development or progress of different diseases.

Using different models of stress, including global brain ischemia or inhibition of UPS, we have shown specific responses of the tissues and cells to the particular stress. In addition, we have shown that mild stress can activate pro-survival cell responses that increase resistance of the tissues and cells to the consequent lethal stress.

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Keywords: UPS, ER, mitochondria, ISR, UPR

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YEAST SURFACE DISPLAY PLATFORM FOR THE SELECTION OF GFP AND mCHERRY NANOBODIES

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Abstract: The yeast surface display is a technique used to display proteins on the surface of yeast cells. It allows the selection and isolation of proteins with specific binding properties, such as nanobodies. Nanobodies or the single-domain antibody fragments derived from camelid species, possess high affinity and specificity for their target proteins and can be used as research tools or potential therapeutics. They offer advantages such as small size, only ~15 kDa binding domain, high stability, and the ability to target hidden or difficult-to-access epitopes.
Our project aims to develop a platform for GFP and mCherry nanodies production, using the YSD approach. The green fluorescent protein (GFP)-nanobody or mCherry nanobody are emerging as a powerful tool for the isolation and cellular engineering of fluorescent protein fusions in many different fields of biological research with specific applications, from cellular imaging to protein analysis.

Keywords: Yeast surface display, *Saccharomyces cerevisiae*, nanobody, GFP, mCherry

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INCLUSIVE GENDER EQUALITY IN THE LIFE SCIENCES. IMPORTANCE OF USING THE FULL POOL OF TALENTS IN BIOCHEMISTRY

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The European Union faces an under-representation of women in the natural sciences, according to statistical indicators from the European Commission's latest publication "She Figures" (She Figures, 2021). The problem of under-representation of women scientists also applies to the field of biochemistry and is strongly reflected in research careers, the delegation of decision-making and management positions, the coordination of research teams, publications in high-impact, prestigious international journals and first authorships. The European Commission, through the science policies of the renewed European Research Area, the "EU Equality Strategy 2020-2025", the "Pact for Research and Innovation in Europe", encourages Member States to actively support women and girls in STEM studies and careers. They should also look for more attractive and creative ways of presenting female role models with successful careers in the sciences to boost girls' confidence in their digital skills and encourage them to study in these areas. The European Commission also urges all relevant stakeholders to address discrimination in recruitment and to consider introducing quotas to ensure better inclusion of women in the sector. Women are more likely to be subjected to gender-based violence, including sexual harassment. Responsibility for a safe and inclusive environment, in line with the "Ljubljana Charter on Gender Equality in Research and Innovation" and “ERA Political Action 5”, is a key role of higher education institutions and research organisations to support students, PhD students and staff.

Keywords: inclusive gender equality, balanced reserch and innovation, women carieer progression, ERA Action 5, ending gedner based violence in academia.

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ENZYME ACTIVITY ASSAYS BY MALDI-TOF MASS SPECTROMETRY

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Abstract: Enzyme activities are measured for different purposes starting from a simple enzyme detection to kinetic experiments with substrates and inhibitors. Common ways include spectroscopy, electrochemistry and liquid chromatography. The use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an alternative, which offers high speed, sensitivity, possibility of automation, and cost-effectiveness. As an advantage, it may additionally reveal reaction intermediates, side products or measure more enzymes at once. Either an internal standard or ratiometric approach are used to overcome the inherent inhomogeneous distribution of analyte and matrix in the sample spot, which otherwise results in a poor reproducibility. In our work, several enzyme activity assays based on MALDI-TOF MS have been developed.

The protease Zmp 1 from *Mycobacterium tuberculosis* is a virulence factor important in mycobacterial pathogenesis. It has been considered as a promising target in the development of antituberculotics. MALDI-TOF MS was employed in our laboratory to assess the inhibition rates of synthetic hydroxamates. Alpha-cyano-4-hydroxycinnamic acid (CHCA) was used as a matrix and the IC50 inhibition constants were obtained from the ratio of the reaction rates for inhibited and control reactions. Copper-containing diamine oxidases (EC 1.4.3.22) oxidize their substrates to the corresponding aminoaldehydes, ammonia and hydrogen peroxide. Aminoaldehyde dehydrogenases (EC 1.2.1.19) oxidize the above-mentioned aminoaldehyde products to amino acids using $NAD(P)^+$ as a coenzyme. Both enzymes thus participate in polyamine metabolism. Polyamines have been recognized as important regulators, which are involved in diverse growth and developmental processes (e.g. cell proliferation and differentiation) and in responses to environmental stress. MALDI-TOF MS was applied to measure the kinetic parameters k_{cat} and K_m for various natural and synthetic substrates of pea seedling amine oxidase and compared successfully with those obtained by a standard spectrophotometric method. CHCA was used as a matrix. The measured compounds included putrescine, cadaverine, agmatine, spermidine, histamine, and some others. Pea seedling aminoaldehyde dehydrogenase and two plant aldehyde dehydrogenases of the class 7 were analyzed by MALDI-TOF MS in their reactions mixtures with NAD^+ and synthetic amino acid-derived aminoaldehyde substrates for the possible reaction products. Again, the matrix was CHCA and tryptamine served as an internal standard. The presence of characteristic peaks in the acquired mass spectra allowed to evaluate the substrate properties of the studied compounds and confirm their oxidation to carboxylic acids.

MALDI-TOF MS is definitely a fast and reliable tool to assess enzyme activities. The biggest advantage is its versatility in providing specific substrate and product signals, which is reflected in a large scale of possible applications.

Keywords: activity assay, amine oxidase, enzyme, mass spectrometry, protease

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PRODUCTION OF RECOMBINANT PEROXIDASES, THEIR CHARACTERIZATION AND POTENTIAL USES

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Abstract: Oxygen is critical for the aerobes to carry out their physiological processes. However, in accordance with the use of oxygen, the so-called reactive oxygen species (ROS) develop. Molecular targets of ROS include proteins, DNA, but also lipids. Even the first organisms had to adapt to environmental influences, and peroxidases together with catalase were the only enzymes for detoxification and reduction of ROS. Peroxidase, like catalase, is among the ubiquitous and unique enzymes that split hydrogen peroxide into water. At the same time, the oxidation of substrates takes place, especially phenols and anilines, which are electron donors in the given reaction.

Peroxidases can be divided into two groups: A) heme peroxidases, which have a protoporphyrin IX prosthetic group, and B) non-heme peroxidases, which are less abundant. Phylogenetically, this large group is divided into several superfamilies and families.

Peroxidases represent a class of industrially important biocatalysts due to their catalytic speed and high stability. To date, they have found use in a wide range of industries, including the textile industry, biosensors, corrosion, micromotors, polymers, biopolymers, the food industry, and medicine and pharmaceuticals.

In our laboratory we focus on production of peroxidases with interesting properties mostly using *E. coli* as a production host. Hyperthermophilic catalase from archaeon is the first example. For this enzyme is typical that the catalase activity has a high turnover number, which leads to an extremely high ratio of catalase activity to peroxidase activity, which in this respect this catalase-peroxidase resembles rather typical catalases. The temperature optima for individual activities are 80 °C for peroxidase and 70 °C for catalase activity, and thanks to these properties, this enzyme is an exceptional source of research. Other examples include a bacterial dye-decolorizing peroxidase, hybrid peroxidases and fungal peroxidases. Due the fact that peroxidases act upon many various substrates (exact physiological substrates are unknown) production of a range of peroxidases from different sources may be beneficial for industrial uses.

Recombinant proteins, and especially peroxidases, represent an interesting group of enzymes, and their heterologous production is an irreplaceable part in almost all branches of industry.

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Keywords: peroxidases, recombinant protein, heterologous expression

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BIOSCIENCE STUDY PROGRAMS AT THE FACULTY OF NATURAL SCIENCES CU AND DEPARTMENT OF MOLECULAR BIOLOGY

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Abstract: There will be presented and discussed current situation in Bioscience study programs at the Faculty of Natural Sciences CU in Bratislava, including Bc. degree study programs (Biology, Medical Biology, both held in Slovak language, and Biological Chemistry, which is held in English). The presentation will also include more detailed information about MSc. and PhD. degree study programs Molecular Biology and Biotechnology, which are provided at the Department of Molecular Biology.

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Keywords: *Molecular biology, biotechnology, teaching*

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UNVEILING THE POTENTIAL OF LIQUID BIOPSY: NON-INVASIVE CANCER SCREENING FOR EARLY DETECTION AND PREVENTIVE, PREDICTIVE AND PERSONALIZED MEDICINE

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Abstract: Nowadays, precise genomic characterization and cancer screening demonstrate that conventional molecular-based methods are being replaced by high-throughput and more affordable massively parallel sequencing technologies. Their integration into routine clinical practice is expected to be essential in managing severe pathologies. Most of them, including cancer, present minimal to no phenotype in the early stages. Therefore, early detection enabling effective disease treatment remains a challenge for contemporary medicine. Analysis of circulating extracellular DNA (cfDNA) has shown potential in identifying genomic alterations that could represent early neoplastic changes. Here, we focus on liquid biopsy-based strategies using shallow coverage whole-genome sequencing data from plasma samples at an individual's genome level. We discuss the results obtained by applying bioinformatics strategies to analyze patients with different malignancies, focusing on colorectal cancer patients. We investigate correlations between copy number variants and clinical-pathological annotations from CRC patients and evaluate their functional impact on CRC-related signaling pathways. Additionally, we propose a model to assess the fragment size distribution of cfDNA, which demonstrates high predictive value when stratifying cohorts of CRC patients and healthy individuals from the Slovak population. We also point out the high diagnostic potential of not only these sequencing metrics and genomic attributes. Our results thus advance liquid biopsy in preventive, predictive and personalized medicine to the level of promising non-invasive cancer screening.

Keywords: liquid biopsy, cfDNA, massively parallel sequencing, cancer screening

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NARCOS AND BEYOND: NATIONAL SURVEILLANCE OF SARS-CoV-2 AND OTHER PATHOGENS

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Abstract: More than three years have passed since the World Health Organisation announced the global pandemic of SARS-CoV-2 virus with major impact on healthcare, economy worldwide. Genomic variability of the coronavirus led to emergence of novel phylogenetic variants with increased infection rate and partial evasion from specific immunity. Therefore, one of key tasks in pandemic management has been the genomic surveillance of clinical COVID-19 cases in all countries. Although our group had sequenced the first clinical cases as early as March 2020, systematic weekly sequencing has started until March 2021, almost a year later. The sequencing efforts in Slovakia are coordinated by the national Public health authority. Based on a lower number of cases in early 2021, the original planned sample throughput was 500 samples per week and four laboratories were involved. With the emergence of Delta and Omicron variants the required sample number has grown almost four times. This led to an increase in the rate of analyzed samples in participating laboratories and the addition of new sequencing laboratories. To ensure reliable per case analysis of clinical samples in six Illumina based sequencing laboratories, metadata transfer automation, unified variant calling and interpretation and batch upload to GISAID and ENA database as well as fast reporting to TESSY database, we developed an web based integrated information system for sequencing management, data and metadata transfer and automated batch reporting and uploading to relevant databases. Uniquely tuned in-house variant calling pipeline allowed us to unify the analysis of all samples from Slovakia. The name of the system is NarCoS. Aggregate results visualizations are used for reporting purposes. As the SARS-CoV-2 is currently fading in intensity, the development and surveillance efforts are refocusing on the inclusion of other pathogens, such as influenza viruses or RSV. There is a growing importance of monitoring of wastewater for the viral pathogens due to limited availability of clinical samples. NarCoS has recently been successfully validated by ECDC external quality audit.

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AN UNUSUAL COMPOSITION OF MITOCHONDRIAL PYRUVATE DEHYDROGENASE COMPLEX OF DIPLONEMIDS, WIDESPREAD MARINE PROTISTS

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Abstract: Pyruvate is a key molecule in cell metabolism produced by glycolysis and entering the tricarboxylic acid cycle after its conversion to acetyl-coenzyme A by a mitochondrial complex of pyruvate dehydrogenase (PDH). Related complexes, those of 2-oxoglutarate dehydrogenase (OGDH) and branched-chain ketoacid dehydrogenase (BCKDH), share evolutionary origins with PDH and their appearance dates back to the last eukaryotic common ancestor. We analyzed sequence data from diplonemids, one of the most abundant oceanic protist groups, to identify the composition of their mitochondrial dehydrogenase complexes. Whereas the OGDH and BCKDH complexes exhibit a standard make-up comparable to other eukaryotes, the PDH complex is compositionally unique, harboring an E1-type subunit of prokaryotic origin. In *Paradiplonema papillatum*, a model diplonemid, we show that this atypical subunit appears to be functional as it is present, based on mass spectrometry data, at a considerable level in the mitochondrion. Immunolocalization of tagged PDH subunits also confirmed mitochondrial localization of complex. The overall PDH activity in this marine flagellate is comparable with related euglenozoans. Moreover, the specific PDH activity is responsive to nutrient availability and can not be replaced by the related OGDH complex.

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Keywords: diplonema, mitochondria, pyruvate dehydrogenase

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EPIGENETIC LANDSCAPE IN PROGNOSIS OF UVEAL MELANOMA

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Abstract: Uveal melanoma (UM) is the most common primary intraocular malignancy and accounting for 5% of all primary melanoma cases. Despite outstanding advances in understanding the genetic background of UM development and prognosis, this disease has high metastatic death rate and no effective treatment. Therefore, understanding the metastatic process and its drivers is of utmost importance. Chromosomal aberrations include several UM-specific rearrangements (chr.1, 3,6,8) and divide patients into two prognostic groups, which poor prognostic UM patients (Class 2) develop metastasis in 50%, mostly in the liver (89%).
Epigenetic changes are important events driving cancer progression, therefore to clarify the extent of DNA methylation deregulation and its utility as a reliable prognostic biomarker and for characterisation of methylation events associated with UM prognosis, is needed.
Transcriptomic and DNA methylation landscapes in 25 high- and low-risk UMs followed by validation analysis in 58 samples discovered high content of methylation events. Among 2,262 differentially expressed genes discovered in UM samples differing in metastatic risk, 60 were epigenetic regulators, mostly histone modifiers and chromatin remodelers. A total of 44,398 CpGs were differentially methylated, 27,810 hypomethylated, and 16,588 hypermethylated in high-risk tumors. By integrative analysis, 944 differentially expressed DNA methylation-regulated genes were revealed, 635 hypomethylated/upregulated, and 309 hypermethylated/downregulated, covering epigenetic regulators, mostly histone modifiers and chromatin remodelers. Aberrant DNA methylation in high-risk tumors was associated with the deregulation of key oncogenic

pathways such as EGFR tyrosine kinase inhibitor resistance, focal adhesion, proteoglycans in cancer, PI3K-Akt signaling, or ECM-receptor interaction.
Nine genes *HTR2B*, *AHNAK2, CALHM2, SLC25A38, EDNRB, TLR1, RNF43, IL12RB2*, and *MEGF10* with the best integrative analysis values were selected for validation and all demonstrated excellent risk group prediction accuracies (AUCs ranging between 0.870 and 0.956). Moreover, *CALHM2* hypomethylation and *MEGF10, TLR1* hypermethylation, as well as two three-gene methylation signatures, Signature 1 combining A*HNAK2, CALHM2,* and *IL12RB* and Signature 2 A*HNAK2, CALHM2,* and *SLC25A38* genes, correlated with shorter overall survival. **EDNRB, IL12RB2, CALHM2** and *RNF43* play important role in interaction with immune and stromal cells in tumor microenvironment and AHNAK2 correlate with infiltration of immune cell subpopulations such as $CD8^+$ and $CD4^+$. These findings highlight the critical role of DNA methylation aberrancy in driving transcriptomic changes associated with poor prognosis and has the potential to serve as reliable prognostic biomarkers, underscoring the clinical relevance of DNA methylation analysis in UM.

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AGING AND THE ROLE OF MAMs IN THE PROTECTION OF THE POSTISCHEMIC HEART

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DISEASE DIAGNOSTICS BY ANALYSIS OF GLYCANS

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Abstract: The most common post-translational modification of proteins is glycosylation, which affects most membrane and serum proteins. Glycans are information-rich molecules that increase the solubility and stability of glycoproteins, and their main role is participation in cell adhesion or signalling. The presence of a given glycan structure cannot be deduced from any template and it is a great challenge to reliably analyse changes in glycan structure. Changes in the structure of glycans accompany various pathological processes, such as oncological or autoimmune diseases. Analysis of the exact structure of glycans is technically and time-consuming (pre-concentration and purification steps, enzymatic release of glycans) using a combination of instrumental techniques. Therefore, the analysis of glycans by integrating methods with mass spectrometry for the analysis of minor glycoproteins present in real samples is often problematic. The technology developed by us enables glycoprofiling of poorly represented oncomarkers in a minimal amount of blood serum in situ (without glycan release), within a simple two-step protocol based on magnetic nanoparticles in a common ELISA plate. This technology has been successfully applied to the early diagnosis and monitoring of disease effectiveness in prostate cancers with high accuracy.
Analysis of glycans can not only refine the diagnosis of prostate cancer, but it can be used for diagnostics of other diseases. Part of the lecture will be devoted to the way in which new biomarkers are validated and what are the hot trends in the diagnosis of various types of cancer, e.g. by exosome analysis.

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Keywords: diagnostics, glycans, lectins, exosomes

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RATIONAL PROTEIN ENGINEERING DRIVEN BY KINETICS

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Abstract: Kinetic analysis is a powerful tool providing invaluable information about individual steps and the mechanism of action of biologically and industrially relevant biomolecules, namely recombinantly produced enzymes. Rigorous characterization provides better understanding of their molecular principles and especially identification of the rate-limiting step. This information is an essential base for the improvement of recombinantly produced proteins by the methods of rational protein engineering. Removing the major bottleneck and enhancing the overall effectivity of proteins is the critical prerequisite for their industrial application which is otherwise hardly profitable. This approach was applied on three model recombinant proteins: (i) staphylokinase, clinically interesting thrombolytic protein activating cleavage of pathological blood clots; (ii) haloalkane dehalogenases, commercially utilized in the HaloTag label technology; and (iii) luciferases, bioluminescent enzymes emitting visible light implemented in diagnostics. Kinetic analysis of staphylokinase provided an extended mechanism with not yet characterized off-pathways and limitations, allowing the determination of reliable catalytic activity value previously underestimated by the factor of 10,000. Removal of the identified limitation provided a staphylokinase variant with 7-fold improved affinity and 10-fold improved selectivity towards its physiological partner. Characterization of haloalkane dehalogenases revealed that the catalytic histidine mutation in the HaloTag technology should be substituted with asparagine to yield effective, highly irreversible labelling. Finally, investigation of *Renilla*-type luciferases allowed deciphering their catalytic mechanism which proceeds via a superoxide radical and requires (de)protonation events during the catalytic cycle. A comparison of luciferase variants kinetic parameters confirmed that the flexible regions are essential for effective substrate binding and light emission. Engineering these critical aspects provided luciferase variants with increased substrate affinity, modified colours of light emission, and a 100-fold prolonged half-life of the emitted light. Collectively, the results of the kinetic analysis provide understanding of proteins structure-function relationship and essential information for their improvement using the rational design approach. The generated recombinant protein variants represent attractive proteins for biotechnological application.

Keywords: enzyme kinetics, protein engineering, luciferase, staphylokinase, haloalkane dehalogenase

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FAMILY GH13 TREHALOSE SYNTHASES WITH A C-TERMINAL MALTOKINASE – AN *IN SILICO* STUDY

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Abstract: Trehalose synthase (TS), a member of the subfamilies GH13_16 and GH13_33 of the α-amylase family GH13, catalyses the interconversion of maltose and trehalose. Some of the GH13_16 TSs may contain a maltokinase (MaK), suggesting their role in the 4-step metabolic pathway of glycogen biosynthesis recently revealed in some bacteria. Of total 5,933 GH13_16 members, 3,347 non-redundant TS sequences were selected and analysed for the presence of the eventual MaK domain succeeding the TS. A group of 1,425 enzymes containing the complete MaK fused to TS was collected; the MaK domains being compared with true MaK and analysed in detail. While most of the fused enzymes contain a standard MaK with conserved catalytic residues and a binding site for maltose, some contain just a MaK-like domain that is probably not active due to mutations in the MaK catalytic residues and/or in the binding site for maltose. Analysis of the linker region connecting MaK to TS suggests that the MaK domains in fused enzymes are likely to be longer than those found in true MaKs. The present bioinformatics analysis could thus help in studying the role the MaK and/or MaK-like domains may play in conjunction with GH13_16 TSs.

Keywords: CAZy database; trehalose synthase; maltokinase; fused enzymes; bioinformatics

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PACLITAXEL-INDUCED NEUROPATHY: BIOMARKER INSIGHTS FROM GLYCOPROTEOMICS

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Abstract: Paclitaxel, a potent chemotherapeutic agent, is extensively used in the treatment of breast cancer, a prevalent cancer type worldwide. Despite its effectiveness, a major challenge in its clinical application is its connection to peripheral neuropathy. This debilitating side effect can significantly impact patients' quality of life, often presenting as numbness, tingling, or pain in the hands and feet, limiting everyday activities, and frequently necessitating dose reduction or treatment discontinuation.

At present, the ability to predict which patients are more likely to develop paclitaxel-induced peripheral neuropathy is insufficient, and no medications are currently available to treat or suppress this neuropathy. This inability to forecast this adverse effect hampers the clinicians' ability to personalize treatment plans, potentially compromising treatment efficacy and patient quality of life.

To address this critical gap, our clinical study aims to explore the blood serum glycoproteome in patients before paclitaxel treatment who had acquired paclitaxel neuropathy (N = 21) or whose treatment had not caused these issues (N = 11). Healthy patients (N = 20) will also be included to assess the glycoproteomic footprint of breast cancer. Glycoproteomics, which involves the study of changes in protein glycosylation, has the potential to uncover biomarkers that could indicate disease states and responses to treatment. Glycosylation can influence several protein functions, including stability, solubility, or its affinity with interaction partners. We hypothesize that specific glycosylation patterns could indicate an increased risk of developing peripheral neuropathy following paclitaxel treatment. On top of studying glycoproteome, we would like to measure blood serum cytokines in both patient groups, due to their strong correlation with neuropathy development.

After our in-depth glycoproteomic characterization and cytokines measurement, we intend to use machine learning techniques to determine if a specific combination of these biological features could form the basis of a predictive model for paclitaxel-induced neuropathy.

Keywords: Paclitaxel, Neurotoxicity, Glycoproteomics, Prediction, Machine Learning

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BIOCHEMICAL STRATEGIES FOR HEALTHY AGING – FROM SIRT-FOOD TO ALKALINE AND MAGNESIUM SUPPLEMENTATION

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Abstract: During recent years biomedicine has revealed several important mechanisms for the aging process. Fasting has often been smiled at as an anti-aging strategy. However, recent research has shown that fasting-induced ketosis and subsequent autophagy is of utmost importance in avoiding premature aging. Aside of fasting also food components like SIRT-activators induce autophagy. The beneficial effects of ketosis are widespread, but it is often forgotten that ketones induce an acidosis. Negative effects of this mild metabolic acidosis like inhibition of lipolysis or induction of unspecific pain problems can be avoided by alkaline supplementation. Mild acidosis also might induce urinary loss of magnesium. Generally, low magnesium status is a risk factor especially for diseases of the aged. Fasting induced ketosis and ingestion of SIRT food is effective in anti-aging if accompanied by enough alkalines and magnesium.

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MAGNETIC PARTICLES IN BIOAFFINITY INTERACTIONS FOR IMPLEMENTATION IN THE DIAGNOSIS OF ONCOLOGICAL DISEASES BASED ON GLYCANS

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Abstract: The social pressure to find solutions for issues related to oncological diseases is constantly increasing. In 2020, 19.3 million new cancer cases and approximately 10 million cancer-related deaths were recorded. One of the ways to improve patient prognosis and detect cases in the early stages is through early diagnosis. In this study, an assay for detection of the cancer biomarker Thomsen-nouvelle (Tn) antigen on the ELISA plates format was designed and developed. The effects of size and funcional groups of magnetic beads (MBs) on the specific sensitivity of the bioaffinity interaction were studied. Four different MBs were used in the study, i.e. carboxy-modified MBs of 250 nm, 500 nm, 1000 nm and 2800 nm. In order to evaluate which MBs are the best suited for detection of the analyte anti-Tn antibodies, sensitivities of detection (slopes from calibration curves) were calculated. Then, we evaluated the effect of size on the specific sensitivity of detection of anti-Tn antibodies in order to understand the immobilisation process on nanoscale. We obtained a limit of detection (LOD) (0.31 ± 0.01) ng mL- 1 or (2.10 ± 0.04) pM for analyte detection. In addition, the optimal assay configuration was highly selective and enabled reliable detection of the analyte in human serum with a recovery index in the range of 102 -104%. New approaches to gather information about the overall state of the body focus on studying exosomes. Extracellular vesicles (EVs) that reflect the content of parent cells and the surrounding extracellular matrix can be a potential source of new biomarkers. From the perspective of glycans, it is possible to monitor pathophysiological changes in the body through aberrant glycosylation (fucosylation, branching of glycans, presence of polysialic acid), which is one of the signs of oncological disease development. Our focus is on isolating exosomes using affinity methods, meaning the utilization of antigen-receptor interactions and corresponding antibodies, with an emphasis on the tetraspanin receptor family. Our objective is to isolate tissue-specific exosomes present in blood serum using magnetic particles, the surface of which will be modified with antibodies, followed by glycoprofiling through various methods.

Keywords: glycans; anti-glycans antibody, biomarkers; magnetic beads; detection, extracellular vesicles
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IN VITRO SYSTEMS FOR INVESTIGATING METABOLIC SULFATION OF NATURAL POLYPHENOLS

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Abstract: Natural polyphenols from plant-derived foods are extensively metabolized after absorption by phase II conjugation enzymes that produce mainly glucuronides and sulfates. The authentic metabolites of polyphenols are needed as standards for metabolic studies and they should also be included in biological activity studies because human tissues can be exposed both to the parent compounds and to their biotransformation products. Our experiments have shown that sulfated metabolites of natural polyphenols, namely, flavonoids and flavonolignans, can be successfully prepared using aryl sulfotransferase from *Desulfitobacterium hafniense* and *para*-nitrophenyl sulfate as a sulfate donor (Roubalova *et al*. Bioorg. Med. Chem. 2015, 23, 5402; Valentova *et al*. Int. J. Mol. Sci. 2018, 19, 2349). To find out whether these sulfates can be produced in human tissues, several *in vitro* systems with sulfation activity can be used, including *i*) primary cell cultures such as isolated human hepatocytes (Vrba *et al*. J. Pharm. Biomed Anal. 2018, 152, 94), *ii*) human cell lines such as hepatoma HepG2 cells (Vrba *et al*. Phytother. Res. 2012, 26, 1746), and *iii*) cytosolic fractions isolated from human tissues such as the liver and intestine. Finally, a series of commercially available recombinant human sulfotransferases (SULTs) can be used to identify the enzymes responsible for the metabolic sulfation of natural polyphenols (Vrba *et al*. Metabolites 2020, 10, 329). The subcellular systems, *i.e.*, the cytosolic fractions and recombinant SULT enzymes, require 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as a sulfate donor. In contrast, cellular systems do not require the cofactor PAPS and allow complete metabolic profiles of the tested compounds to be obtained. The biotransformation of selected flavonoids using the above systems is presented.

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Keywords: Metabolism, sulfation, sulfotransferase, flavonoid.

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PROTEIN GLYCOSYLATION: SYNTHESIS, SIGNIFICANCE IN HEALTH AND DISEASE, AND ITS SPECIFIC DETECTION BY LECTINS

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CATALASE IS DETRIMENTAL FOR *LEISHMANIA* VIRULENCE (WITH NOTES ON EVOLUTION OF CATALASES IN *TRYPANOSOMATIDAE*)

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Abstract: Catalase is one of the most abundant enzymes on Earth. It decomposes hydrogen peroxide, thus protecting cells from dangerous reactive oxygen species. The catalase-encoding gene is conspicuously absent from the genome of most representatives of the family Trypanosomatidae. The exceptions are monoxenous relatives of *Leishmania* spp., and representatives of the genera *Blastocrithidia*, *Obscuromonas*, and *Vickermania.* In this work, we expressed the *Leptomonas seymouri*-derived catalase from the *Leishmania mexicana beta-tubulin* locus using a novel bi-cistronic expression system, which relies on the 2Apeptide of *Teschovirus A*. We demonstrated that catalase-expressing parasites are severely compromised in their ability to develop in insects, to be transmitted and to infect mice, and to cause clinical manifestation in their mammalian host. Taken together, our data support the hypothesis that the presence of catalase is not compatible with the dixenous life cycle of *Leishmania*, resulting in loss of this gene from the genome during evolution of these parasites. To complement these data, we ablated a catalase-encoding gene from the *Leptomonas seymouri* genome and established an add-back, where catalase was overexpressed from the 18S rRNA locus of this flagellate. Our study demonstrated that parasites' development and infectivity *in vivo* (in *Dysdercus peruvianus* model) depends on the expression level of this enzyme. These studies were further complimented by biochemical characterization of three independently-acquired catalases (of *Blastocrithidia*, *Leptomonas*, and *Vickermania*) *in vitro*, which showed that the enzyme of *Blastocrithidia nonstop* is cyanide-resistance, an unprecedented feature among all investigated monofunctional catalases.

Keywords: catalase, *Leishmania*, Trypanosomatidae, dixeny

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IMPACT OF VITAMIN C SUPPLEMENTATION ON THE EPIGENETIC MODIFICATIONS IN DNA AND MRNA EXPRESSION OF VITAMIN C TRANSPORTER GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA.

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Abstract: Vitamin C is an essential component of many enzymes including TET proteins (ten-eleven translocation) which can play a crucial role in the demethylation DNA process. TET enzymes catalyze the oxidation of 5¬methylcytosine (5¬mCyt) to 5-hydroxymethylcytosine (5¬hmCyt) and further oxidation products. It was demonstrated that ascorbate may enhance generation of epigenetic DNA modifications in cultured cells, acting as a cofactor of TETs during hydroxylation of 5-mCyt. Intracellular level of vitamin C may depend on the efficacy of vitamin C transport into the cell by sodium-dependent transporters (SVCTs), which are capable to accumulate vitamin C against a concentration gradient. Chronic lymphocytic leukemia (CLL) is a common hematological malignancy accounting for roughly 30% of all leukemia and characterized by remarkable clinical heterogeneity. It is suggested that epigenetic changes are relevant for CLL etiology and the disease development.
We have examined CLL patients before and after six months of oral vitamin C supplementation. Expression of genes involved in active vitamin C transport was evaluated using quantitative RT-PCR. We applied ultra-performance liquid chromatography methods with mass spectrometry and/or UV detection for determination of epigenetic DNA modifications and vitamin C concentrations in the blood plasma and within leukocytes.
We found significant increase blood and intracellular levels of vitamin C after oral implementation without alterations in expression of genes encoding vitamin C transporters. Moreover, we noticed differences in the levels of DNA demethylation products before and after supplementation.
Our results suggest that despite the increase in intracellular vitamin C content after supplementation, it does not affect the increase in the level of active demethylation products, which in turn, may indicate a disturbance in the activity of TET enzymes and then in DNA methylation pattern. We did not find that vitamin C supplementation restores levels of TET products in leukocytes of CLL patients.

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PRETOMANID AND DELAMANID AS THE NEWEST PLAYERS AGAINST TUBERCULOSIS

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Abstract: Tuberculosis (TB) is an infectious disease that most often affects the lungs. It is caused by bacterium *Mycobacterium tuberculosis*. TB is an airborne disease that can be spread by coughing or sneezing and is one of the leading causes of death by single infectious agent worldwide. According to the latest World Health Organisation`s 2022 Global Tuberculosis Report, an estimated 10.6 million people fell ill with tuberculosis in 2021, a 4.5% increase from the previous year [WHO, 2022]. There were an estimated 1.4 million deaths among HIV-negative people and an additional 200,000 deaths among HIV-positive people. Treatment of drug-sensitive TB takes 4 - 6 months with up to four different antibiotics, which is difficult for the patient. Currently, one of the biggest problems is the emergence of resistant strains, which are longer and more complex to treat.

Delamanid and pretomanid are two drugs that are used to treat multidrug-resistant or extremely drug-resistant TB. Both are prodrugs that are activated by the F420-dependent nitroreductase Ddn [Singh, R., *et al.* (2008) *Science* 322, 1392]. Delamanid has been used in Europe since 2014 to treat multidrug-resistant TB. Pretomanid is a bicyclic nitroimidazole that was approved for the treatment of extensively drug-resistant TB in 2020 and is indicated in combination with bedaquiline and linezolid, in adults [Conradie F., *et al.* (2020) *N. Engl. J. Med.*, 382:893-902]. Although both drugs, pretomanid and delamanid, are used in TB treatment, their mode of action is still not clear. It was shown that both drugs are effective against replicating, as well as non-replicating mycobacteria [Stover, C. K., *et al.* (2000) *Nature* 405, 96; Matsumoto, M., *et al.* (2006) *PLOS Med* 3, 466]. A crucial element of anaerobic activity of pretomanid is respiratory poisoning through nitric oxide release [Singh, R. *et al.* (2008) *Science* 322, 1392]. In the presence of oxygen, pretomanid and delamanid affect the composition of long chained fatty acids, mycolic acids, in mycobacteria, where a decreased production of their keto- forms and accumulation of hydroxymycolic acids was demonstrated [Stover, C. K., *et al.* (2000) *Nature* 405, 96]. Efforts to find the active metabolites led to discovery, that both drugs form an adducts with NAD contributing to their bactericidal activity [Kreutzfeldt, K. M., *et al.* (2022) *Nat Commun* 13, 2203]. In our work we demonstrated that the effect of pretomanid and delamanid on mycolic acid synthesis is not lethal, and both drugs affect several targets including enzymes involved in the synthesis of cell wall arabinogalactan.

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Keywords: delamanid, pretomanid, mycobacteria, tuberculosis

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WOMEN IN SLOVAK LIFE SCIENCES PROJECTS

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Achieving gender equality is one of the main goals of the new European Research Area (ERA). The European Commission's strategy for equality between women and men for the period 2020-2025 defines the vision, political goals, and measures to achieve concrete progress in the field of gender equality and to achieve goals of sustainable development.
The Slovak Centre of Scientific and Technical Information (CVTI SR), as the national information centre for science, technology, innovation, and education of the Slovak Republic, undertakes to contribute to the fulfilment of the goals by incorporating the perspective of gender equality into the content of science, research, and innovation. CVTI SR within its competence manages several national registers, which process scientific and research data and serve for their support.
The Slovak Information System for Research, Development, and Innovation (SK CRIS), ensures the collection, processing, provision, and use of data from research, development, and innovation supported by public funds. SK CRIS contains a national register of research and development projects, a register of researchers, a register of organisations, information on research results and laboratory infrastructure. Incorporation of the CERIF data format within the SK CRIS enabled the collection of gender data.
The demand for gender equality and equal opportunities for all apply for research teams at all levels. Our analysis relates to the registered research teams in the SK CRIS, i.e., is based on the links of researchers to projects, within the projects register. For the analysis of gender equality in life sciences, the data collection Projects implemented in 2021 was examined.
Research projects registered in the SK CRIS information system are categorised by research area. As part of the categorization of the focus of the projects, it was necessary to identify those categories that belong to life sciences.
The analysis shows that the share of women involved in research projects in general (regardless of the scientific area) in the monitored period was more than 45%. However, if we look at the share of women according to the basic groups of R&D fields, the representation of women begins to differ depending on the selected group of scientific fields.
The share of women in biological sciences roughly corresponds to the share of women in medical sciences and in veterinary sciences and it is the highest among the monitored categories (approx. 62-63%). We can conclude that the involvement of women in research activities in the field of life sciences in Slovakia is above average.

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Keywords: life sciences, research projects, women in science, data analysis

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COMPARATIVE ANALYSIS OF MITOCHONDRION-RELATED ORGANELLES IN ANAEROBIC AMOEBOZOANS

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Abstract Archamoebae is a group of amoeboid protists with free-living or endobiotic life strategies, all inhabiting anaerobic or microaerophilic environments. Instead of aerobic mitochondria, they house mitochondrion-related organelles (MRO) of various metabolic capacities. In this study, we compared predicted MRO proteomes of eight species (six genera) and proposed the scenario of their evolution. The common ancestor of Archamoebae likely possessed a reduced/divergent protein translocation machinery similar to that in extant species. On the other hand, the MRO metabolic capacity decreased lineage-specifically. The glycine cleavage system is widely conserved among Archamoebae, except in *Entamoeba*, probably owing to its role in catabolic function or one-carbon metabolism. Pyruvate metabolism was disposed of in Entamoebidae and Rhizomastixidae lineages, and the sulfate activation pathway was lost in three isolated cases - *Rhizomastix libera*, *Mastigamoeba abducta*, and *Endolimax* sp. A bacterial NIF-type of the Fe-S cluster assembly system was acquired through lateral gene transfer in the common ancestor of Archamoebae and duplicated in the common ancestor of Mastigamoebidae and Pelomyxidae, with one copy participating in Fe-S assembly within MRO. In Entamoebidae and Rhizomastixidae, dual localization of the system in the cytosol and the MRO may in principle allow Fe-S cluster assembly in both compartments. We could not find evidence for changes in the MRO metabolic functions in response to the transition to an endobiotic lifestyle, suggesting that environmental drivers do not strongly affect MRO reduction in this group.

Key words: reductive evolution; mitochondrion-related organelles; anaerobiosis; comparative genomics.

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EXPRESSION AND FUNCTION OF SPECIFIC NEUROPEPTIDE-RECEPTOR PATHWAYS IN TICKS

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Abstract: Ticks are blood-feeding ectoparasites that transmit numerous pathogens from the salivary glands or gut into the vertebrate hosts. These pathogens cause serious diseases in appropriate host animals and humans. To determine regulatory pathways involved in transmission of these pathogens, we examined expression of identified neuropeptides and their receptors in the tick *Ixodes ricinus*. Various molecular approaches revealed expression of several neuropeptides in central neurons innervating the salivary glands, gut, accessory glands and ovaries. Additional regulatory peptides are produced by various types of endocrine cells in the midgut. As expected, these organs showed increased expression of corresponding neuropeptide receptors during feeding and development. Physiological experiments and RNAi approaches revealed specific roles of neuropeptide-receptor pathways in feeding, digestion and reproduction of ticks that are associated with transmission of pathogens.

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Abstracts of posters

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APPLICATION OF LONG-READ SEQUENCING FOR DETECTION OF COMPLEX CHROMOSOMAL REARRANGEMENTS

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Introduction: Complex karyotype (CK) typically involves various, often extensive numerical and structural chromosomal abnormalities. In chronic lymphocytic leukemia (CLL), it represents an established biomarker of adverse outcome. Common methods to detect CK include classical cytogenetics and genomic microarray; additional information may be obtained using next-generation sequencing (NGS). However, all these approaches show low accuracy and sensitivity for detecting complex structural variants at the DNA sequence level. We aimed to explore the ability of long-read sequencing for the precise characterization of complex genomic variants in CLL patient samples.
Methods: High-molecular weight DNA (HMW DNA) was isolated using isopropanol-chloroform extraction from 15 CLL samples with known somatic genomic rearrangements. HMW DNA was fragmented using a 30G needle, followed by size selection to remove fragments shorter than 10 kb using the PacBio SRE Kit. Library preparation was performed using Ligation Sequencing Kit (Oxford Nanopore Technologies, ONT) and sequenced on the MinION and PromethION platforms. Three MinION flow cells and one PromethION flow cell per genome were used for 4 and 11 samples, respectively. Generated sequencing data were aligned to the hg38 human genome reference and breakpoints were detected with the SVIM variant caller.
Results: The coverage achieved using either the MinION or PromethION platform ranged from 10.2 to 11.9× and from 20.5 to 37.3×, respectively. Breakpoints identified using both platforms were compared with available cytogenomic results of classical karyotyping, mFISH and genomic microarray. Most of breakpoints detected by these methods were confirmed by long-read sequencing, however, breakpoints located near centromeres remained unidentified. Our data were further compared to results from other experimental techniques, including short-read whole-genome sequencing, optical genome mapping and Hi-C. We observed breakpoints in known CLL-associated genes, as well as in non-recurring genes affecting cell signaling pathways, uncovering the possible ways how detected abnormalities impacted biological processes in CLL cells.

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Discussion and conclusion: Our study contributes to a better understanding of the structural variants present in complex CLL genomes and their impact on the leukemic cell phenotype. Via confirming previously detected breakpoints using long-read sequencing, we demonstrated the reliability of this approach in characterizing selected chromosomal rearrangements. However, challenges remain in identifying breakpoints in highly repetitive regions. To address this, we intend to analyze our data against the T2T human genome reference, which is expected to improve breakpoint identification in these regions.

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Keywords: complex karyotype, chronic lymphocytic leukemia, long-read sequencing

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EFFECT OF BACTERIAL GROWTH RATE ON OVERPRODUCTION OF LON PROTEASE

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Abstract: Lon protease represents a homo-oligomeric ATP-dependent protease, which is one of the main enzymes of the quality control system of the cellular proteome in all domains of life – Archaea, Bacteria and Eukaryotes. The Lon family is divided into subfamily LonA, which includes bacterial and eukaryotic enzymes, and subfamily LonB, which combines the archaea proteases. In bacteria, Lon protease also fulfils the role of a global regulator, governs functions such as DNA replication and repair, bacterial metabolism, virulence factors, toxin-antitoxin system, stress response and biofilm formation. This work compares the protein productivity of two bacterial platforms based on their different growth rates. We opted for *Escherichia coli* and *Vibrio natriegens* as expression hosts to produce Lon protease from the uropathogenic strain *E. coli* KL53. *V. natriegens* is one of the fastest growing bacteria with a reported doubling time of <10 min, while the shortest generation time of *E. coli* is doubled. In a previous work (results not shown), we focused on the design and preparation of the plasmid construct pET28a-Lon. In first step we transformed BL21(DE3) and Vmax cells with the mentioned plasmid containing the gene for Lon protease, which is under the control of the inducible T7 promoter. After the selection of recombinants, we carried out a series of expressions in Erlenmayer flasks for 4 hours. Cells were cultured at three different temperatures (20°C, 28°C, 37°C) using media with various compositions (LB, LB3, TB, TBv2). Samples taken from 0.-4. hour of expression in Erlenmayer flasks were analyzed by SDS-PAGE electrophoresis and then evaluated by densitometric analysis. To verify the presence of the protein of interest, we additionally performed a western blot for samples from both *E. coli* and *V. natriegens*. In our work, we managed to successfully produce Lon protease in both bacterial expression systems, what was also confirmed by western blot analysis. The relative percentage representation of the target protein was higher in the case of *E. coli* strain BL21(DE3).

This research was supported by the Slovak Research and Development Agency grant APVV-19-196.

Keywords: *Escherichia coli*, *Vibrio natriegens*, Lon protease, heterologous expression

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INVESTIGATION OF THE ROLE OF PA200 INDEPENDENT OF PROTEASOME BINDING AND ACTIVATION

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Abstract: Proteasome activators can interact with the proteasome core particle (20S CP) at one or both ends of the 20S CP to activate its catalytic activity and promote substrate degradation. The well-characterized 19S regulatory particle (RP/19S/PA700) forms the canonical 26S proteasome with the 20S CP which is required for ATP- and ubiquitin-dependent substrate unfolding and degradation. In addition, alternative activators of the proteasome have also been described. The conserved Blm10/PA200 family is present in yeast and mammals, whereas activators of the PA28 protein family are present in higher eukaryotes. Both Blm10 and PA200 mediate specific substrate degradation of proteins such as unstructured proteins and acetylated histones in an ATP- and ubiquitin-independent manner. However, our current understanding of the biology of Blm10/PA200 is still in its infancy and is much less advanced than that of the other proteasome activators.

Recently, we have identified PA200-enriched regions in the genome of SH-SY5Y neuroblastoma cells using ChIP-Seq. We found that PA200 protein peaks are located near transcription start sites. Gene ontology annotation revealed that genes whose promoters were enriched following anti-PA200 ChIP contribute to the regulation of important intracellular processes. Our ChIP-Seq analysis also led to the identification of a putative binding site for PA200 in the genome of SH-SY5Y cells.

We therefore decided to confirm that PA200 actually recognizes and binds to this putative site PA200 in the human genome and that PA200 actively participates in gene regulation.

We have designed and are currently optimizing an in vitro firefly luciferase reporter assay. The results of our work may provide evidence for a novel role for PA200, independent of its binding to the proteasome core and involvement in substrate degradation by the proteasome.

Keywords: Gene regulation, Luciferase reporter assay, PA200, **P**roteasome activator

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ANALYSIS OF BACTERIA AND BACTERIOPHAGES IN WHEY FROM SLOVAKIAN BRYNDZA CHEESE PRODUCTION

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Abstract: Slovakian bryndza cheese is a traditional ewes' milk-based cheese, which is produced in Slovakia. This distinctive cheese is recognized in the European Union by Protected Geographic Indication status (PGI; Commission Regulation 676/2008). Its unique organoleptic properties, in particular taste and aroma, guarantee its high popularity in Slovakia and in neighbouring countries, a stable market position as well as its acceptation as an item of national cultural heritage. A widely successful practice in production of traditional cheeses is the use of natural whey starters. A natural whey starter is a part of the fermented whey from a previous successful production batch, which is kept refrigerated and can be used to speed up the initial fermentation phase of the cheese.
The aims of the project was to study bacterial and bacteriophage composition of whey cultures and to isolate microorganisms potentially useful as starter cultures.
The bacterial composition of whey microbiome was determined by 16S rRNA amplicon sequencing and the predominance of *Lactococcus* genus was observed followed by *Acinetobacter* spp., *Enterococcus* spp. and *Enterobacteriaceae*. Although lactobacilli did not make up a significant proportion of all bacteria, they are known to substantially affect organoleptic properties of Slovakian bryndza cheese. Fifty lactic acid bacteria were isolated from various whey samples and analysed by microbiological and molecular methods. *Lacticaseibacillus casei/paracasei*, *Lactiplantibacillus plantarum/paraplantarum* and *Lentilactibacillus parabuchneri* were the most frequent representatives. The strains were analysed regarding presence of genes encoding defence systems, antibiotic resistance and production of biogenic amines. The best candidates were selected as starters in model cheese production.
As bacteriophages are viruses infecting bacteria, they can infect lactic acid bacteria responsible for cheese fermentation and, in some cases, may cause delay or failure of fermentation. For this reason, we analysed phage composition of whey samples by metagenomic approach. Several predominantly lactoccocal phages were detected in concentrated whey. By cocultivation of whey with *L. fermentum* and *L. brevis* strains, several DNA contigs with homology to lactobacilli phages were determined. The obtained results will facilitate technical development of starter cultures for production of Slovakian bryndza cheese with traditional organoleptic properties.

This work was supported by the project APVV-20-0001.
Keywords: lactic acid bacteria, lactobacilli, microbiome, food

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FORMATION OF DENTAL STEM CELL 3D ORGANOIDS AND OPTIMIZATION THEIR GROWTH CONDITIONS FOR BONE REGENERATION

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Abstract: Bones play an important role in maintaining exercise and protecting organs. Bone defects can cause tremendous damage with long treatment cycles. Therefore, treating bone defects remains one of the main challenges in clinical practice. At present, the method of clinical treatment for bone defects includes non-invasive and invasive therapy. Surgical treatment is the most effective way to treat bone defects, such as using bone grafts and other techniques. In recent years, the rapid development of tissue engineering technology provides a new treatment strategy for bone repair. Bone tissue engineering uses scaffolds, well-integrated cells, and bioactive growth factors to promote bone repair and regeneration, providing an innovative platform for regenerative medicine. The hydrophobicity, pore size, and biochemical structure of the scaffold can affect cell growth and viability.

Mesenchymal stem cells are the most used cells in regenerative medicine and can be obtained from almost every human body tissue. Teeth and the surrounding dental tissues are a natural source of mesenchymal stem cells, which are collectively called dental stem cells (DSCs). The potential for clinical use of these cells lies primarily in their excellent proliferative and differentiation properties, their non-invasive and affordable acquisition. It is precisely for this use that dental stem cells derived from dental pulp, either from adult teeth (DPSCs) or from deciduous teeth (SHEDs), are very promising. In our research, we focus primarily on the osteodifferentiation potential of dental stem cells, which in the future can be applied in the treatment of damaged bones after diseases and injuries.

The aim of the presented work was to optimize conditions for 3D cells, including various combinations of growth factors and optimization of biomaterial components appropriate for our cell type. We monitored the morphological changes of the cells during osteodifferentiation using light microscopy and also specific proteins by fluorescence microscopy.

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Keywords: 3D organoids, dental stem cells, osteodifferentiation

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THE ROLE OF MARIX METALLOPROTEINASES AT PATHOLOGICAL SITUATIONS

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Abstract: Matrix metalloproteinases (MMPs) are enzymes playing an important role in degradation and remodeling of extracellular matrix. Moreover, dysregulation of MMPs activities plays a crucial role in pathophysiology of several diseases such as cardiovascular, neurodegenerative, and metabolic diseases, and cancer. The relation between dysregulation of their function and oxidative stress has been documented. Important role in regulation of MMPs activities/expression play reactive oxygen and reactive nitrogen species, especially superoxide and peroxynitrite.

In our studies, we investigated the role of MMPs in pathophysiology of cardiovascular system diseases and studied their relation to cellular redox signaling. The obtained results pointed to important role of several MMPs (especially MMP-2, MMM-9, and MMP-28) as potential biomarkers of pathological states development. We found increased activities of circulating 72 kDa MMP-2 at pathological conditions associated with both diabetes development and effects of doxorubicin (DOX). Moreover, effects of DOX were in rats associated with up-regulation of 72 kDa MMP-2 activities in left ventricular (LV) cardiac tissue. Similar 72 kDa MMP-2 activation in LV we observed in diabetic ZDF rats. Increased activation of this form of MMP-2 can occur in disease conditions associated with redox imbalance. This was supported by findings that the effects of DOX and diabetes on MMP-2 activation were associated with reduction of total superoxide dismutase (SOD) activities. Especially in the effects of DOX appears to be important, in a relation to MMPs and redox signaling, the involvement of proteins
involved in autophagy regulation. Application of flavonoid quercetin (QCT) led to reversal of negative effects of pathological conditions on MMP-2 and SOD. The obtained data showed that in animals exposed to DOX had application of QCT positive impact on myocardial protection. MMP-28 is the newest identified member of the MMP family and decreases of its protein levels in response to pathological conditions has been documented. Our results showed significant down-regulation of protein levels of MMP-28 in obese diabetic ZDF rats. Application of QCT led to reversal of effects of diabetes on MMP-28.

The observed data suggest that the changes in activation of MMP-2 and protein levels of MMP-28 may have a negative impact on the progression of pathological changes in cardiovascular system induced in consequence of oxidative stress. Application of flavonoid quercetin can mediate the reversal of negative effects of pathological conditions on MMPs.

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MITOCHONDRIAL METABOLISM OF MONOXENOUS TRYPANOSOMATIDS

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Abstract: Members of the family Trypanosomatidae (Phylum: Euglenozoa, Class Kinetoplastea) are unicellular flagellated protists causing serious diseases in humans, agricultural plants and livestock. The best known *Trypanosoma brucei* and *T.cruzi* cause sleeping sickness in Africa and Chagas disease in Latin America, respectively. All pathogenic trypanosomatids are dixenous – they alternate between two hosts, insects and mammals, respectively plants. However, the most trypanosomatids are monoxenous - they have only one host, which is mostly insect. The results of the last period show a surprisingly large variability of their metabolism and show the importance of their study for understanding the fascinating diversity of life. The knowledge gained during this study can also help in the search for potential metabolic sites for the fight against pathogenic species. In our study we focused on biochemical characterization of more than dozen trypanosomatids and comparing them with already described species. We characterize the activities of the enzymes involved in oxidative phosphorylation (OXPHOS), Krebs cycle (KC) and pyruvate dehydrogenase complex using two different approaches – spectrophotometry and in gel staining. We present activities of complexes II, III, IV and trypanosome alternative oxidase (TAO) of OXPHOS and citrate synthase and malate dehydrogenase of KC. Our data confirm quite big differences in enzyme activities between individual monoxenic trypanosomatids.
Supported by grants SK-CZ-RD-21-0038 and VEGA 1/0553/21

Keywords: trypanosomatids, mitochondria, bioenergetic metabolism, enzymes

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CELL DEATH UPON REPEATED ADMINISTRATION OF SULFORAPHANE TO P-GLYCOPROTEIN NEGATIVE OR POSITIVE L1210 CELL VARIANTS.

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Abstract: Cells are capable of developing resistance to multiple drugs (MDR) with different structures and mechanisms of action. The expression of the membrane ABCB1 transporter in neoplastic cells is one of the most common causes of reduced sensitivity to chemotherapy. The most commonly observed molecular cause of MDR is massive cellular drug efflux mediated by drug transporters that are predominantly members of the ABC protein family, in particular ABCB1 transporter (also known as P-glycoprotein). We investigated the effect of a single culture of ABCB1-negative (S) and ABCB1-positive variants of L1210 cells (R and T) in the presence of sulforaphane (SFN).We demonstrated that SFN induces the onset of autophagy more markedly in S cells than in R or T cells. We focused on the effect of the repeated culture of S, R and T cells in SFN-containing media. The repeated cultures increased the onset of autophagy compared to the simple culture, mainly in S cells and to a lesser extent in R and T cells, as indicated by changes in the cellular content of 16 and 18 kDa fragments of LC3B protein or changes in the specific staining of cells with monodansylcadaverine. We conclude that SFN affects ABCB1-negative S cells more than ABCB1-positive R and T cells during repeated culturing. Changes in cell sensitivity to SFN appear to be related to the expression of genes for cell-cycle checkpoints, such as cyclins and cyclin-dependent kinases.

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Keywords: sulforaphane; multidrug resistance; ABCB1 transporter; autophagy

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METHODS FOR THE DETECTION OF CAUSAL MUTATIONS CAUSING CANINE CARDIOMYOPATHY TO OBTAIN INDIVIDUALS SUITABLE AS MODELS OF HUMAN CARDIOMYOPATHY

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Abstract: The dog genome is a valuable tool for studying hereditary diseases. Dogs serve as spontaneous models for many human genetic disorders, facilitating the identification and study of disease-associated loci. Dogs share the same environment as humans, which affect the development and course of diseases similar or the same in human. Cardiomyopathies (CM) are classified into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC). HCM and ARVC are primarily caused by mutations in genes encoding sarcomere or desmosome proteins, while DCM involves over 250 associated genes. Genes, such as *RBM20*, *TTN*, *PLN*, *YARS2*, *DSP*, and *RYR2*, have been identified as causing DCM or CM in both dogs and humans. Dogs provide models for studying human heart disease, even for genes not yet identified in humans, like the *PDK4* gene, regulating energy metabolism in the heart and skeletal muscles. The RNA-binding motif protein 20 (*RBM20)* plays a crucial role in regulating alternative splicing by binding to pre-mRNA of cardiac genes, including titin (*TTN)*. The phospholamban (*PLN*) regulates calcium levels, and *YARS2* is involved in calcium release during cardiac muscle contraction. Mutations disrupt their function, leading to mitochondrial dysfunction in humans and CM with juvenile mortality (CJM) in Belgian Shepherds. Our work aims to design methods for detecting causal mutations responsible for the development of DCM in certain dog breeds. The analysis involves a set of predisposed individuals to identify carriers. By appropriately crossing carriers (only for research), obtaining a lineage of individuals with the mutation in a homozygous state is possible. These naturally occurring animal models serve as suitable models for acquiring additional knowledge about the pathophysiology of the disease, studying disease progression, evaluating the impact of various therapies, examining the effectiveness of different drug treatments, pharmacokinetics, etc. We have designed and implemented fast, cheap, and simple methods (PCR-RFLP, PCR-ACRS, ARMS) for the detection of causal mutations in the mentioned genes. These methods can detect mutation carriers or individuals with the mutation in the state.
In a set of 40 Rhodesian Ridgeback and 31 Doberman, we analysed the presence of a 16 bp in intron 10 of the PDK4 gene. We identified 10 heterozygotes 3 Doberman and 3 Rhodesian

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Ridgeback with the mutation in a homozygous state. We have also designed methods for the detection of the following mutations in predisposed breeds: missense variant C/T in TTN gene (Dobermann), RBM20: c.2472_2493del (Schnauzer), and c.1054G>A in YARS2 gene (Belgian Shepherd).

This study was supported by the Operation Program of Integrated Infrastructure for the project, UpScale of Comenius University Capacities and Competence in Research, Development and Innovation, ITMS2014+: 313021BUZ3, co-financed by the European Regional Development Fund.

Keywords: Cardiomyopathies, DCM, dog model, mutation

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CASE REPORTS IN MEDICAL BIOCHEMISTRY COURSE

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Abstract: Case studies are widely recognized as valuable and effective supplemental tools for postgraduate and senior undergraduate medical studies. They provide a practical and in-depth approach to learning, allowing students to apply their knowledge in real-world situations.
At the UPJŠ in Košice, Faculty of Medicine, the integration of case studies into the teaching of medical biochemistry played an important role following the complete transition from face-to-face education to online education during the summer semester of 2021 due to the COVID-19 pandemic. The prepared case studies have been specifically adapted to meet the needs of general medicine students enrolled in the subject Medical Biochemistry 2, which primarily focuses on the biochemistry of organs. Even after the return to face-to-face teaching, the case studies retained their value as optional supplementary material.
Students often perceive medical biochemistry as one of the most challenging subjects and generally respond positively to the presentation of case studies. They particularly appreciate the practical approach of the case studies and find them helpful in understanding complex connections in real-life scenarios.
It is important to note that the use of case studies in teaching medical biochemistry can be controversial, especially if the clinical detail level exceeds the second-year students' knowledge level. Striking a balance is crucial, ensuring that the "dose of clinical messages" aligns with the student's level of understanding. Appropriately worded and carefully structured case studies, tailored to medical biochemistry students' specific needs and knowledge level, serve as highly stimulating learning resources. Furthermore, they promote a positive and enthusiastic approach to the study of medical biochemistry, fostering the development of critical thinking and problem-solving skills, which are essential for future healthcare professionals.
We are currently developing the KEGA project "CASE-PORTAL", which promises to be a significant addition to the traditional methods of teaching medical biochemistry.

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Keywords: case report, education, motivation

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FUNGAL GLYCOSIDASES FOR BIOTRANSFORMATION OF FLAVONOIDS AND GALACTOOLIGOSACCHARIDES

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Abstract: The α-galactosidase (EC 3.2.1.22) and α-L-rhamnosidase (EC 3.2.1.40) are exotype carbohydrases which catalyze the cleavage of terminal α-linked galactosyl and rhamnosyl residues from a wide range of substrates, including linear and branched oligosaccharides, polysaccharides, pectin, flavonoids and synthetic substrates. α-Galactosidase have a number of biotechnological applications: in beet sugar industry these enzymes are used to remove raffinose from beet molasses and to increase the yield of sucrose; they are also used to improve the gelling properties of galactomannans to be used as food thickeners and to degrade the raffinose family sugars (raffinose, stachyose and verbascose) in food and feed materials such as soya meal or soya milk. α-L-Rhamnosidase can be used to improve the quality of food products and can be involved in the production of many drugs and their precursors. The purpose of this work was to isolate two glycosidases from a new producer of *Penicillium restrictum* and to investigate their properties. Gel filtration on Toyopearl HW-55, Sepharose 6 B, and ion-exchange chromatography on DEAE Toyopearl 650m were used for purification of the enzymes from micromycete cultural liquid. The activity of α-L-rhamnosidase was determined using the Davis method, naringin was used as a substrate. α-Glalactosidase activity was determined using synthetic nitrophenyl substrate. Naringin, rutin, neohesperidin, hesperidin, narirutin, raffinose, stachyose, galactomannan (0.5-1 mM), and *p*-nitrophenyl-α-L-rhamnose, *p*-nitrophenyl-α-D-galactose (1 mM) were used to assay the enzymes substrate specificity. The changes in the concentration of substrates were measuring by HPLC. The specific activity of purified α-L-rhamnosidase was 27.8 U/ml, and α-galactosidase — 49.1 U/ml. The enzymes were homogeneous according to gel filtration on Sepharose 6B and had a molecular weight of 50 kDa and 17 kDa. Both glycosidases had high activity and stability in the pH range of 4.0-6.0 and thermal optimum at 60 and 65 ºC. It was shown that glycosidases have the high stability at 20°C to the action of metalloproteases (elastase and collagenase) and serine proteases (proteinase K, pronase E, and trypsin). There was no decreasing in activity of three α-galactosidases upon standing them in a solution with proteases at 20 ° C for 24-48 hours. α-Galactosidase from *P. restrictum* has broad substrate specificity. The hydrolysis rate of raffinose, stachyose and galactomannan was 133, 116 and 27 μmol/min/ml, respectively. Also the efficiency of biotransformation of α-1,6 and α-1,2-rhamnosylated flavonoids by α-L-rhamnosidase *P. restrictum* was demonstrated. Advantageous functional properties and substrate specificity of studied enzymes for modifying rhamno- and galactoglycosides suggest their broad-range applicability for food and animal feed processing, as well as the pharmaceutical industry.

Keywords: α-galactosidase, α-L-rhamnosidase, *Penicillium restrictum, flavonoids, galactooligosaccharides*

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NOVEL TWO-DOMAIN LECTIN FROM BACTERIUM PHOTORHABDUS LAUMONDII

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Abstract: Lectins, saccharide-binding proteins, play a key role in the pathogenic processes of diverse bacteria. High interaction specificity enables the recognition of the host cell and adhesion to it. Rather low binding affinity is compensated by a quite strong avidity effect, as lectins are usually polyvalent. *Photorhabdus* spp., entomopathogenic bacteria, produce various lectins, a few of which have been already characterized.
In my work, I focus on producing and characterizing a novel two-domain lectin from *Photorhabdus laumondii*. First of all, bioinformatical analysis was performed. A synthetic gene was inserted into a production vector and the recombinant protein was produced by E. coli. After production optimization, several methods were used to characterize the binding properties of the recombinant protein, e. g. batch method, and hemagglutination.

Keywords: lectins, *Photorhabdus laumondii*, molecular cloning, recombinant protein production

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WHOLE GENOME ANALYSIS OF *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM FOOD PROCESSING ENVIRONMENTS

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Abstract: *Listeria monocytogenes* is a bacterial pathogen responsible for the life-threatening disease listeriosis. The most common cause of listeriosis is considered to be ingestion of food contaminated by *L. monocytogenes*. *L. monocytogenes* comprises four separate deep-branching lineages, which, from an evolutionary viewpoint, could be considered separate species. The lineage I clones CC1, CC2, CC4 and CC6 are reported to be associated with human disease, while lineage II clones CC9 and CC121 are strongly associated with food and food processing environments. Whole-genome sequencing (WGS) based subtyping demonstrates several advantages over traditional subtyping approaches, including enhanced discrimination, prediction of antimicrobial profiles, attribution of transmission sources, and so it shows great potential for microbial food-safety surveillance.

In cooperation with the Food Research Institute we have analyzed 27 strains isolated from meat factory and sheep dairy farm. The collection consisted of persistent strains repeatedly isolated from the same factory as well as of sporadic transiently present strains. Based on MLST method, these strains were classified into 13 sequence types. Most of the strains belonged to sequence type ST-14 (n=18), followed by ST-9 (n=3), ST-2 (n=2) and ST-394 (n=2). The average genome length was 2.9 Mb. Using the cgMLST method with a panel of 1748 genes of the core genome, we divided the same strains into 33 unique groups. We have identified genes playing a role in virulence and persistence of *L. monocytogenes* strains in food processing environment. We found that persistent strains of ST14 isolated from a meat factory carry in their genome genes encoding for a resistance to benzalkonium chloride, which is the basis of disinfectants used in the food industry. Two strains belonging to ST-6 carry *Listeria* pathogenicity island 3 (LIPI-3). We have also identified prophage content in *L. monocytogenes* strains and 72 various prophages prophages were detected. The number of prophages varied from one to five per bacterial strain. Five prophages was present in ST 451 sporadic strain isolated from a sheep dairy farm. The most frequent site of prophage integration was into tRNA genes. Other integration sites were identified in genes encoding for a competence transcription factor and an esterase. Prophages integrated into tRNA genes were the second most frequent. Their genome size was on average 40 kbp and they were complete prophages with an average number of 60 genes. They could be further divided into

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12 subgroups and were also relative to several prophages published in databases. The most frequent group identified in the genome of *L. monocytogenes* strains was group A which was present in all analyzed strains followed by group F (19 strains) and group I (14 strains). The presence of endolysins in prophages was also studied as these enzymes have the potential to be used as an antimicrobial agent for the biological control of *L. monocytogenes* growth in food products and food processing environments.

Keywords: endolysin, Listeria monocytogenes, persistence, prophage, WGS

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CHROMATIN CONFORMATION ANALYSIS REVEALS GENOME STRUCTURAL AND FUNCTIONAL COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Abstract:
Introduction: The spatial chromatin organization in the nucleus has been recognized to be essential for fundamental cell processes. Disruption of its hierarchical structure can be caused by chromosomal rearrangements, which, especially in cancer cells, lead to aberrant molecular signaling, affecting tumor phenotype. We aim to study altered genome structure in chronic lymphocytic leukemia (CLL), the most common type of leukemia of adults in the Western world. We are particularly interested in the complex karyotype (CK) that is known to be associated with poor clinical outcome. We assume that CK plays a key role in chromatin structure disruption, consequently affecting CLL behavior.
Methods: Chromatin crosslinking was carried out on B cells separated from the peripheral blood of 5 CLL patients with CK. Further sample processing was performed using a Micro-C kit (Dovetail Genomics) and NEBNext Ultra II DNA Library Prep Kit (New England Biolabs). Sequencing libraries were deep-sequenced to >300M reads (2x150 bp mode on NovaSeq, Illumina). Obtained data were pre-processed using the pipeline created by Dovetail Genomics. Whole-genome contact maps were visualized with the Juicebox tool. For copy number variation (CNV) and structural variation (SV) detection, we applied bioinformatic tools EagleC and NeoLoopFinder. Finally, Micro-C data were compared with other conventionally used genome-wide methods (i.e., mFISH and CytoScan HD array) to evaluate the benefit of integrating the Micro-C technique for CK analysis.
Results: We obtained data allowing for a detailed analysis of DNA interactions with 50 kb resolution. Genomic rearrangements identified in contact maps as interchromosomal interactions were primarily compared with mFISH results. CNV plots representing gains and losses of genomic material were compared with array-based results. Overall, using the chromatin conformation analysis, we identified abnormalities detected by conventional methods. Additionally, we identified quantitative and qualitative differences related to the method

resolution, sensitivity, and other molecular (e.g., epigenetic) mechanisms. Detailed analysis and interpretation of interacting genome regions is ongoing.

Conclusion: By analyzing chromatin interactions in CLL samples, we want to contribute to the knowledge of leukemia genomic complexity and related abnormal signaling. Our data show that the Micro-C method has great potential for detecting SVs, including balanced and cryptic translocations that remain hidden from other techniques. The main advantage of the method lies in the additional information about interactions of the chromatin in specific regions, which has not been possible to analyze by other methods.

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Keywords: chronic lymphocytic leukemia, chromatin conformation, complex karyotype

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PLASMA CHOLINESTERASE POLYMORPHISMS MONITORING IMPORTANT EITHER FOR THE CHOICE OF MUSCLE RELAXANT OR FOR THE BLOCKADE REVERSAL

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Abstract: Postoperative residual curarization (PORC) is characterized as the presence of muscle fatigue, exhaustion, or insufficiency as a result of the use of neuromuscular blocking agents (NMBA) with a prolonged postoperative effect. It is associated with hypoxia, respiratory failure and increased perioperative morbidity. Symptoms such as weakness, hypoxia or the inability to cough can occur relatively often, but are rarely associated with residual curarization. The incidence of PORC can be up to 50%. This can be avoided by choosing an agent that is not metabolized by cholinesterase, however the reversal of the effect of neuromuscular block is also affected by the presence or absence of a genetic variant of cholinesterase. Moreover, polymorphism in plasma cholinesterase gene has been shown to be associated with tumorigenesis, metabolic risk factors or Alzheimer disease. The participants underwent surgery under general anesthesia using a non-depolarizing relaxant - rocuronium with an intermediate duration of action. All participants were extubated in the operating room and transported to the recovery room accompanied by an anesthesiologist and an anesthesiologist nurse, where they were immediately connected to a vital signs monitor. Patients were given oxygen through a face mask and were followed by a train-of-four ratio (TOF) - Watch muscle relaxation monitor. We investigated the frequency of two polymorphisms in the plasma cholinesterase gene causing change in enzyme activity and metabolism of applied drugs, known to reduce its activity by approximately 30%. Initial results suggest a relatively high incidence of plasma cholinesterase variant K risk allele (18.75%). When comparing with need for medications used for rocuronium neuromuscular block reversal, three patients carrying K variant were used to end blockade by sugammadex. However, five variant K carriers were treated by neostigmine. At this point, number of patients is small. However, further characterization of the lacking informations about genetic background of changes in plasma cholinesterase activity within Slovakia may allow for easier decision-making in clinical practice when selecting alternative neuromuscular blocking and also reversal agents.

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Keywords: butyrylcholinesterase, cholinesterase, neuromuscular blocker, polymorphism, residual curarization

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ENHANCING THE PRODUCTION OF THE THYROID-LIKE PEROXIDASE ENZYME IN *ESCHERICHIA COLI* BY APPLYING THE CARBOHYDRATE-BINDING MODULE AS A FUSION PARTNER

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Abstract: Novel functions or improved activities engineered into proteins in a variety of ways must have sufficient solubility to retain their bioactivity. Nonetheless, inactive protein clumps are commonly observed in *Escherichia coli* during heterologous protein expression. The efficient production of soluble and functional recombinant proteins is one of biotechnology's primary objectives. Under saturated protein folding machinery conditions, protein misfolding and protein aggregation are promoted. To impede the development of inclusion bodies due to their effectiveness in the soluble expression of target proteins, the convenience of use, and the feasibility of purification, fusion tag technology has been widely used. As a result, scientists have consistently created unique fusion tags to increase the expression potential of valuable proteins in *E. coli*. For the soluble production of targeted heterologous proteins Thyroid-like peroxidase (BbePox-1) in *E. coli*, a novel fusion tag containing carbohydrate-binding module 66 (CBM66) was developed. In this survey, BbePox-1, which is isolated from *Branchiostoma belcheri* considered for heterologous expression in *E. coli* (BL21 DE3). The nucleotide sequence of the mentioned enzyme is engineered into the pCBM66 expression vector, amplified, and transformed into BL21 DE3 cells alongside the T7 promoter-based system to investigate the expression output and solubility. The ultimate objective of this investigation is to achieve high levels of peroxidase enzyme expression in microbial strains, especially in *E. Coli* expressing strains. According to this, the effect of the fusion tag, which is employed in protein expression, can be examined. In the subsequent steps, if a sufficient quantity of the target protein is successfully expressed, the desired fusion will be cleaved from the target protein by protease means such as enterokinase, and the target protein will then be purified by applying chromatographic methods. In addition, various aspects of the fermentation process that can influence the expression, such as temperature, shaking speed, culture media, etc., will be examined, and optimization in production will be carried out. The characterization of the expressed and purified proteins will take place in further steps, and additional crystallographic studies will be carried out.

Keywords: Heterologous Expression, Solubility, Peroxidase, Fusion Partner

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EFFECT OF ATP-DEPENDENT POTASSIUM CHANNELS ON MITOCHONDRIAL DYNAMICS IN AN *IN VITRO* MODEL OF NEURODEGENERATION

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Abstract: ATP-sensitive potassium channels (KATP) are pleiotropic molecules with significant effect on membrane potential and associated calcium homeostasis. Beside the cytoplasmic membrane, their presence was identified on inner mitochondrial membrane as well. Therefore, KATP are essential in numerous physiological as well as pathological processes. More than two decades, their participation in the process of neurodegeneration is still the subject of intensive research. Until these days, the consensus about their exact effects in specific type of neurodegeneration was not reached yet. At least in survey of parkinson´s type of neurodegeneration, beneficial as well as harmful effects of glibenclamide/diazoxide were observed. Namely, documented contrary results strongly depends on chosen model, in which KATP modulators were used. Our working hypothesis about the reason of this phenomenon is based on presence of differences in mitochondrial activity throughout the used experimental models of neurodegeneration. In actual study, we use KATP modulators in control and pathological conditions induced by mitochondrial complex I inhibition in SH-SY5Y cells. KATP antagonist (glibenclamide) or agonist (diazoxide) was added to growth medium in control conditions or simultaneously with rotenone for the 24h. Differences in mitochondrial activity were induced by all-trans retinoic acid, which stimulated the process of differentiation of SH-SY5Y cells. Adult neurons could be characterized by more matured mitochondrial network with promoted oxidative phosphorylation in comparison to standard form of SH-SY5Y with neuroblastoma origin. In the methodological approach, the accent was put on the detection of changes in mitochondrial morphology and dynamics. For this purpose, fluorescence microscopy was used as a pivotal tool. Data obtained by analysis of mitochondrial images were supported by parallel analysis of energy metabolism mediators in growth medium by nuclear magnetic resonance. Level of mitochondrial respiration was evaluated by oximetric measurement. Regarding the morphological features of mitochondrial network observed in differentiated vs non-differentiated SH-SY5Y cells, we identified quite different character of response to treatment by rotenone as well as KATP modulators. After the modulation of KATP activity, the most significant effect on mitochondrial dynamics was observed in the case of KATP opening by diazoxide for 24 h during rotenone treatment. However, the character of diazoxide effect was also strongly influenced by the process of differentiation. According to obtained results we can

conclude that the activity of mitochondrial network inside the cell could be one of the crucial factors regulating the character of the KATP effect in selected model.

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Keywords: KATP modulators, mitochondrial dynamics, neurodegeneration

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PRODUCTION OF MUTANT TAQ DNA POLYMERASE IN VIBRIO NATRIEGENS EXPRESSION SYSTEM

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Abstract: DNA polymerases are enzymes extensively used in various applications, including the amplification of specific DNA regions through the polymerase chain reaction. These enzymes have found utility in diverse fields such as basic research, diagnostic testing, pharmaceutical industry, and biotechnology. Our main focus was production of mutant recombinant Taq DNA polymerase in expression system *Vibrio natriegens*. Taq polymerase, known for its exceptional thermostability, is widely utilized in molecular biology techniques, biotechnology, clinical diagnostics, and sequencing. In our study we used *Vibrio natriegens* as a production organism. *Vibrio natriegens*, a Gram-negative bacterium, offers several advantages as a production organism in biotechnology and industrial applications. It achieves high biomass yields quickly, has a versatile metabolism and is easy to cultivate. Successful recombinant strain construction was achieved using the pET28a vector and designed synthetic gene encoding mutant Taq DNA polymerase. Target gene amplification was carried out using PCR, followed by the creation of the final construct via restriction cloning. Subsequently, we accomplished the transformation of *Vibrio natriegens* - strain Vmax. We performed expression of the target protein using the shake flask expression method under different conditions (cultivation temperature, growth medium, etc.). We were able to produce substantial amounts of the target protein using shake flask expression, although we encountered low solubility. In our upcoming research, we will focus on optimizing the production of the soluble mutant recombinant Taq DNA polymerase.

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Keywords: Taq DNA polymerase, *Vibrio natriegens*, protein production, expression

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EFFECT OF METALLIC SALTS ON ACTIVITY OF MODIFIED CATALASE-PEROXIDASE AFKATG

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Abstract: In recent years the term green chemistry attracted much traction in an effort to reduce energy intensity of some chemical production techniques and to curb global carbon emission. Lucrative alternative to traditional processes arises from the use of enzymes which are biodegradable, non-toxic and very efficient at catalysing their respected reaction. One promising group of enzymes are catalase-peroxidases which are bi-functional enzymes acting as catalase and peroxidase concurrently. Catalase activity is contra productive in production setting as it depletes its co-substrate hydrogen peroxide. For this reason, we modified recombinant haem catalase-peroxidase AfKatG isolated from archaeon *Archaeoglobus fulgidus* to remove its catalase activity. In our work we study the effect of metallic salts addition to reaction mixture with o-Phenylenediamine as substrate. These findings are beneficial as we can modulate AfKatG activity for practical applications.

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Keywords: green chemistry, catalase-peroxidase, o-PDA

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2,3-DEHYDROSILYBIN PROTECTS CARDIOMYOBLASTS AGAINST HYPERTONIC CYTOTOXICITY IN TEMPERATURE DEPENDENT MANNER

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Abstract: Hypertonic stress is represented by any increase in osmolarity above 270-295 mOsmol/L and results in cell shrinkage, aggregation/misfolding of diverse proteins or cell death. Several pathological conditions such as ischemia, septic shock and dehydration, as well as therapeutic interventions, e.g. mannitol treatment, are associated with osmotic changes. 2,3-dehydrosilybin, a natural compound from polyphenol group, shows a number of positive effects on human tissue. It is particularly known for its hepatoprotective effect or its ability to protect the heart muscle from ischemia in the form of preconditioning. *In vitro* experiments are usually carried out in incubators under physiological conditions, however, there are situations where non-physiological conditions cannot be avoided, e.g. microscopic experiments. This may result in very significant changes in cell behaviour between experiments performed inside and outside the incubator. Therefore, we tested whether temperature affects the effect of 2,3-dehydrosilybin on the H9c2 cardiomyoblast cell line under hypertonic conditions. Neutral red experiments revealed temperature- and dose-dependent protection against hypertonic stress in H9c2 cell line. Regardless of temperature, cells under hypertonic conditions died at a similar rate, whereas 2,3-dehydrosilybin was protective, more so at room temperature. To exclude the possibility of a non-specific effect related to the solubility of 2,3-dehydrosilybin, we determined the protective effects of mono- (3-*O*-Me, 7-*O*-Me and 20-*O*-Me) and di-methylated (3,7-*O*-Me, 3,20-*O*-Me and 7,20-*O*-Me) derivatives of 2,3-dehydrosilybin. The data showed that all tested methylated derivatives, except 3,7-di-*O*-methyl-2,3-dehydrosilybin, act similarly to the parent compound. Finally, we revealed that the mechanism of action of 2,3-dehydrosilybin is likely related to modulation of membrane water permeability. Limited water permeability is associated with a decrease in the level of endoplasmic reticulum stress, which is related to the protective effect.

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Keywords: hypertonic stress, H9c2, 2,3-dehydrosilybin.

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BIOTECHNOLOGICAL PRODUCTION OF RECOMBINANT PROTEINS WITH THERAPEUTICAL CHARACTER IN YEAST EXPRESS SYSTEMS

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Abstract: The biotechnological production of therapeutical proteins is a widely used as a part of the pharmaceutical industry in modern times. Faster, cheaper, but also more massive production in some organisms is only part of the advantages offered by this branch of the biotechnology. However, we are still limited by the number of organisms that are generally suitable for production. The most commonly used organism is the traditional bacterium *E. coli*, in which production is cheap and fast, but the bacterium may have a problem with post-translational modifications, which are necessary for a large number of therapeutics. The opposite case is eukaryotic cell cultures, which carry out post-translational modifications, but production in them is often expensive. Therefore, in this work we decided to compromise and use the yeast *C. utilis*, which combines the advantages offered by the above-mentioned organisms. According to the FDA, it is generally considered as safe, its rapid growth and utilization of various carbon sources combined with post-translational modifications make it a suitable candidate for use in the production of proteins of various nature.

In our work, we focused on the secretion of proteins into the culture medium, which is the first step for making the production of recombinant proteins cheaper. With the created plasmids, which were stably inserted into the yeast genome by homologous recombination, we tested the suitability of using the secretory signals, invertase and α-mating factor in this production system. The invertase secretion signal was proven as an unsuitable for our purposes, even in the case of optimized codon usage. To detect the efficiency of secretion, we chose the enzyme α-glucosidase, the activity of which can be measured by the chromogenic substrate PNPG (4-nitrophenyl β-D-glucopyranoside).

In the next part of the work, we focused on the cleavage of the secretory signal by the serine protease enterokinase, which is anchored in the Golgi apparatus membrane with the direction into the lumen. Enterokinase is naturally found in the digestive system of humans and other animals, where it is formed by cells of the duodenum and participates in the digestion of food. As a protease often used in biotechnology, it specifically cleaves the DDDDK sequence after lysine without leaving a residue at the N-terminus of the protein. For this purpose, we created plasmids that manage the anchorage of the light chain of human enterokinase (hEK_L) into the Golgi apparatus membrane and also plasmids that would manage correct detection of the eneterokinase activity in the lumen of the Golgi apparatus. Anchoring will ensure specific cleavage of the secretory signal

of the secreted protein in the yeast *C. utilis* and thus facilitate the purification and finalization of the therapeutic.

Keywords: *Candida utilis,* enterokinase, secretion, production system

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E. COLI BACTERIOPHAGES USEFUL FOR THERAPY OF URINARY TRACT INFECTIONS

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Abstract: Urinary tract infections represent one of the most common problems in clinical medicine. Around 150 million people become infected with this infection every year. The primary cause of community-acquired UTI is the Uropathogenic *Escherichia coli* (UPEC) (about 80– 90%). The World Health Organization (WHO) has declared antimicrobial resistance as one of the greatest threats to human health. UTIs are becoming one of the difficult to treat infections because of the widespread of antibiotic resistance mechanisms among the members of the family *Enterobacteriaceae*, including *E. coli*. The resistance is mainly present on plasmids encoding extended-spectrum β-lactamases (ESBLs) which rapidly spread resistance to third-generation cephalosporin as well as other antibiotics. Currently, the most commonly recommended therapy for UTIs are antibiotics. However, with the increasing rates of bacterial resistance, alternative therapies should be considered to reduce the burden of these common infections. Many promising approaches are being developed, phage therapy is one of the alternative treatment options. Using bacterial phages for therapy has drawn increasing attention in recent years. Phages are viruses that attack bacteria to complete their life cycle. Phage therapy can be defined as the application of strictly virulent phages for the purpose of lysing specific bacterial pathogen which is causing clinical infection. To increase the host spectrum and reduce the likelihood of developing phage-resistant strains of bacteria, cocktails composed of multiple phages are most often used in therapy.

Our team characterized 17 *E. coli* bacteriophages with antibacterial therapeutic potential. Phage cocktail consisting of four phages of the family *Straboviridae* and two of the family *Autographiviridae* was prepared. Phages showed high adsorption to *E. coli* strains, 72-98% in five min interval. The one step growth curve of all phages showed similar parameters (7.5-15 min latent period and 15-30 min whole cycle). The host range was characterized in a panel of 77 clinical strains of *E. coli*, 72% of the tested strains were susceptible to phage infection, these were mainly strains of the B2 and D phylogroup. The inhibition effect of the cocktail was evaluated on five *E. coli* strains. The phage cocktail was able to lyse four strains in LB as well as in artificial urine medium. We can conclude that bacteriophages can be used as a useful strategy for the treatment of infections caused by multi-drug resistant uropathogenic *Escherichia coli* strains.

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Keywords: Uropathogenic *Escherichia coli,* phage therapy, infection.

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MITOCHONDRIAL RESPONSE TO DIFFERENT ROTENONE DOSAGE IN TOXIC *IN VITRO* MODEL

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Abstract: A large part of in vitro models of neurodegeneration is currently based on the inhibition of mitochondrial functions, as one of the causes of neurodegenerative diseases. Rotenone inhibits the mitochondrial complex I and is generally used as a toxic model to study Parkinson´s disease. The data obtained by several up-to-date studies notify us about other rotenone targets inside the cell. Also, its effect seems to be graded by concentrations higher than those necessary for total inhibition of mitochondrial complex I. The imaging of living cells significantly expands the possibilities of microscopic analysis. The use of mitochondrial probes allows the monitoring of mitochondrial functions and the evaluation of the changes in mitochondrial footprint in the cells.
MATERIAL AND METHODS: The SH-SY5Y cells were cultured according to manufacturer instructions. Pathological conditions were induced by inhibiting mitochondrial complex I with the rotenone (Sigma) at concentrations of 50 nM and 10 μM for 2h. Changes in the selected model were detected using a fluorescence confocal microscope LSM880 (Carl Zeiss, Germany). As a probe for mitochondrial network and ROS visualization, Mitotracker Red FM (Invitrogen) and CellROX probe (Invitrogen) were used. Mitochondrial respiration and potential were also detected in living cells using O2K respirometer equipped with a fluorescent LED-2 module (HR FR O2K Oroboros, Austria). Dynamic changes in the mitochondrial skeleton were evaluated in Image J software.
RESULTS AND CONCLUSION: According to the mitochondrial respiration measurement (O2K), we confirmed mitochondrial respiration of complex I is completely blocked already immediately after 50nM rotenone treatment. These results were simultaneously supported by the fluorometric measurement of the mitochondrial potential performed with a safranin fluorescence probe. The low dose of the rotenone (50nM) as well as the further addition of toxin to 10 μM concentration reflects largely reduced mitochondrial potential. The following analyses were aimed at the correlation of the fluorescence results of the MitoTracker Red FM with the fluorescence of the ROS-sensitive probe CellROX. Obtained data demonstrate a strong colocalization of MitoTracker Red FM with CellROX fluorescence probe, especially in high doses of rotenone. Except that, the change of fluorescence intensity of both used probes reveals strong dose dependence with no probable relation to discharged membrane potential. We also found the changes in the mitochondrial skeleton among the selected groups with the reflection of the rotenone dose. Present results strongly support the idea, that effect of rotenone treatment in toxic models is not prominently propagated by complex I inhibition.

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Keywords: fluorescent probe, microscopy, mitochondria, potencial, rotenone

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INVESTIGATING THE REQUIREMENT OF THE CydDC TRANSPORTER FOR THE CYTOCHROME BD OXIDASE ACTIVITY IN MYCOBACTERIUM TUBERCULOSIS

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Abstract: Tuberculosis (TB) is one of the leading causes of human death from an infectious disease worldwide with more than 1.2 million deaths and 10 million new TB cases each year[1]. The remarkable metabolic flexibility of the human pathogen – *Mycobacterium tuberculosis*, requires the administration of novel antituberculosis drugs targeting both replicating and non-replicating bacteria. The electron transport chain in *M. tuberculosis* represents an attractive target space for the development of new antitubercular drugs[2]. *M. tuberculosis* requires oxygen for its growth and possesses two oxygen-dependent terminal respiratory oxidases – cytochrome bc_1-aa_3 oxidase (encoded by *qcrCAB* and *ctaBCDE*) and alternative cytochrome *bd* oxidase (encoded by *cydA* and *cydB*). Dual inhibition of these two terminal oxidases is bactericidal for replicating and non-replicating *M. tuberculosis*[3,4]. Downstream *cydA* and *cydB*, there are two genes encoding a heterodimeric ABC-type transporter CydDC. Recently, it was proposed that its homolog in *E. coli* serves as a transporter of heme important for the assembly of cytochrome *bd*-type oxidases in *Escherichia coli*[5]. However, its biological function and catalytic activity in mycobacteria remain unclear. Here we used CRISPR interference (CRISPRi) to examine the importance of CydDC for the function of cytochrome *bd* oxidase in *M. tuberculosis*. We showed that dual transcriptional repression of *qcrB* and *cydD* has a similar lethal phenotype as dual repression of *qcrB* and *cydA*, suggesting that expression of *cydDC* is required for the viability of *M. tuberculosis in vitro* when cytochrome bc_1-aa_3 oxidase is inactivated. High-resolution respirometry revealed that transcriptional depletion of *cydDC* reduced the oxygen consumption in inverted membrane vesicles isolated from *M. tuberculosis* CRISPRi knockdown indicating the requirement of CydDC for the proper mycobacterial respiration. Moreover, the inactivation of CydDC boosts the antibiotic potency of the cytochrome bc_1-aa_3 inhibitor Q203 in *M. tuberculosis* cells. Our data thus provide evidence for the functional relationship between mycobacterial cytochrome *bd* oxidase and CydDC transporter and highlight the alternative mode for the dual inactivation of oxygen-dependent terminal oxidases in *M. tuberculosis*.

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Keywords: *Mycobacterium tuberculosis*, respiration, CydDC, ABC-type transporter, heme

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FURTHER STUDIES ON THE MECHANISM OF MYCOBACTERIAL GALACTAN EXPORT

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Abstract: Despite ongoing efforts, tuberculosis (TB) remains a global health concern as one of the leading causes of death by a single infectious agent. The cell wall core of *Mycobacterium tuberculosis*, causative agent of TB, is a highly complex and heterogenous structure composed of covalently linked peptidoglycan, heteropolysaccharide arabinogalactan and long-chain mycolic acids which form an outer membrane. Its biosynthesis is a well-known and validated drug target as exemplified by isoniazid and ethambutol – two of the four first-line antituberculosis drugs used in the treatment of TB. While many enzymes involved in the construction of the mycobacterial cell wall have been thoroughly characterized, mechanisms by which the individual components of arabinogalactan are assembled are not fully understood. Recently, an ABC transporter WzmWzt has been shown to export galactan precursor across the plasma membrane into the periplasmic space[1]. Transcriptional silencing of WzmWzt in *Mycobacterium smegmatis* using CRISPR interference caused accumulation of an aberrantly long galactan polymer in the cytoplasm, pointing to a possibility of its export being coupled to its synthesis. Since inhibiting ABC transporters is a viable strategy in current drug development landscape, further insight into mechanism of galactan export has the potential to uncover a novel drug target to aid in the battle with TB.

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Keywords: tuberculosis, mycobacteria, cell wall, galactan, transport

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OVERPRODUCTION OF THE AURICIN-SPECIFIC ACTIVATOR Aur1P INDUCED TYPE I POLYKETIDE SYNTHASE PRODUCT STREVERTENE A

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Abstract: Streptomycetes are dominant producers of bioactive natural products with a broad range of biological activities. A large number of these products belong to polyketides. These structurally diverse compounds are synthesized by repeated decarboxylative condensation from acyl-CoA precursors by a polyketide synthase (PKS). Three types of PKSs are known so far. Type I PKSs (so-called modular PKSs) are large multifunctional enzymes organized into modules with the essential domains (AT, KS, ACP) and non-essential domains (KR, DH, ER). Each module is responsible for catalyzing one cycle of polyketide chain elongation in a non-iterative manner. They are responsible for the biosynthesis of reduced polyketides such as macrolides, polyethers and polyenes. Type II PKSs (so-called aromatic PKSs) are multienzyme complexes acting iteratively. They include three basic subunits (KSα, KSβ, ACP) and other subunits (KR, ARO, CYC), which participate in the formation and modification of the nascent polyketide chain. Furthermore, various tailoring oxygenases, reductases, methylases, and glycosyltransferases generate the final bioactive polyketide. They are responsible for the biosynthesis of aromatic polyketides, which belong to seven structural types: pyranonaphthoquinones, tetracyclines, angucyclines, anthracyclines, tetracenomycins, aureolic acids, pradimycin-type polyphenols. Type III PKSs (also known as chalcone synthase type) are homodimeric enzymes that act iteratively, but unlike the previous types, they are independent of ACP. They are responsible for the biosynthesis of small aromatic polyketides (i. e. chalcone, stilbene). We previously identified the type II PKS biosynthetic gene cluster (BGS) *aur1* in *Streptomyces lavendulae* subsp. *lavendulae* CCM 3239, which was responsible for the production of antibiotic auricin. Interestingly, auricin is transiently produced in a narrow time interval after entering the stationary phase. This unusual pattern of auricin production is the result of its complex regulation by several regulators, Aur1P, Aur1O, Aur1R, Aur1PR3, and Aur1PR4, whose genes are located in the BGC. Structural analysis of auricin revealed that it has interesting structural features. It is modified with D-forosamine and contains a unique spiroketal pyranonaphthoquinone-like aglycone similar to griseusin. Auricin production is low and tightly regulated. To improve its yield, we placed the *aur1PO* operon, encoding key auricin-specific activators, under the control of strong *kasOp** promoter. The resulting strain produced a 1.5-fold higher amount of auricin compared to the WT strain. However, it overproduced 11-fold other secondary metabolite strevertene A, which belongs to the reduced polyene polyketides synthesized by type I PKS. It suggests an interesting cross-regulation of auricin-specific regulators with other BGC.

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Keywords: antibiotics, auricin, strevertenes, *Streptomyces*

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SELF-ASSEMBLING OF SPIDER SILK PROTEIN CONJUGATED WITH BIOLOGICALLY ACTIVE DNA-APTAMERS

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Abstract: Amyloid fibrils are a chiral protein-based system, formed through the self-assembly of β-sheet aggregates into twisted or helical ribbons. Alongside pathological amyloid proteins, functional amyloids fulfil roles in physiological processes in lower organisms, e. g. spiders. Recombinant spider silk protein, able to self-assembly into highly organized structures, has shown high potential for the use in biomedical as well as technological applications based on advantageous properties of the processed materials showing biocompatibility, biodegradability and the absence of immune response[1]. In this study we aimed to prepare DNA-spider silk protein bioconjugates using recombinant spider protein eADF4(C16) derived from the natural dragline protein fibroin 4 (ADF4) of the European garden spider *Araneus diadematus*. The recombinant protein provides the possibility of site-specific chemical modification and subsequent binding of oligonucleotides[2]. As the model oligonucleotides we chose TBA15 and TBA29 known for binding thrombin with different effect. Bioconjugates were synthesized through catalyst-free „click"-chemistry reaction of the modified protein at its N-terminus with azide and the 5´-end-modified oligonucleotides with dibenzocyclooctyne. The accuracy of modifications and subsequent conjugation was verified using high-performance liquid chromatography, mass spectrometry and native gel electrophoresis. As a next step, we aimed to characterize the TBAx-eADF4(C16) bioconjugates in terms of their ability to self-assembly in the presence of different ions using absorbance measurements, circular dichroism and atomic force microscopy. As demonstrated previously for spider silk protein, the presence of phosphate ions triggered also the self-assembly of TBAx-eADF4(C16) into fibrils.[1] However, the kinetics of TBAx-eADF4(C16) bioconjugates was affected by presence of Na^+/K^+ ions. Na^+ ions exhibit higher potential to affect self-assembly than the K^+ ions. The obtained data demonstrate that the bioconjugates TBAx-eADF4(C16) preserve their ability to self-assembly into fibrils and maintain the same properties as naturally occurring cross-β fibrils. Our observations indicate that there is a great potential for using the spider silk protein-DNA bioconjugates to prepare highly organized structures with the ability to bind biologically relevant agents, such as thrombin, which could lead to the practical applications, for example as the drug delivery systems or biosensors.
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[1]Humenik. M., et al. 2020. Mater Today Bio 6, 100045.
[2]Humenik, M., et al. 2014. Nano Lett 14, 3999–4004.

Keywords: recombinant spider protein, bioconjugates, self-assembly, click chemistry

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METABOLIC PATHWAY ANALYSIS IN GENETIC ASSOCIATION STUDY OF DIABETIC RETINOPATHY

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Abstract: The study of multifactorial diseases as diabetic retinopathy is widely based on the analysis of candidate gene variants and genome-wide association analyzes (GWAS), which have become a central method for studying the genetic component of complex diseases. However, a major limitation of the GWAS approach is the mapping of causal variants with a small population frequency and a small effect, which can only be identified in analyzes of large groups, often reaching several thousand tested individuals, which significantly increases the laboratory and financial demands of such studies. Moreover, the conventional approach of GWAS analyzes uses the non-coding, often intergenic single nucleotide (SNP) polymorphisms, which linkage to the true functional variants may not be revealed. Thus, the NGS approach, often focusing on the exome, is used in recent association studies to the possible direct identification of the causal variant. Despite the use of these advanced high-throughput DNA analyses, the genetic factors still remains a question, which is probably mainly due to the multifactorial nature of the studied diseases, participation of variants of small effect, and the small number of samples in individual studies. One of the approaches for elucidating the molecular pathogenesis is the use of the simultaneous analysis of variants belonging to the same metabolic pathways. Metabolic pathway analysis thus may combine the effect of multiple variants of small effect and may increase the power of the study with the test of rare variants that were not recognized in the primary association study. In our study of 46 diabetic patients (with and without retinopathy) we performed the exome analysis with an average reading depth of the target sequences of 150x. We observed total of more than 100,000 variants with a minimum coverage of 20 reads, of which 3613 variants, in over 1000 genes, showed significantly different allelic representation between the two groups of patients (with p -value $\leq$ 0.05). Subsequent over-representation analysis revealed metabolic pathways involved in O-glycosylation, metabolic pathways of cellular organization and components, metabolism of collagen, lipids, plasma lipoprotein, selenium, and others. Metabolic pathway analysis combines the weak effects of multiple variants and increases the power of information mainly in association studies conducted on small number of samples.

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INFECTION OF THP-1 DERIVED MACROPHAGES BY COXIELLA BURNETII BACTERIUM

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Abstract: *Coxiella burnetii* is an intracellular bacterial pathogen that causes human infections of clinical and public health relevance. Like many other bacterial pathogens, *C. burnetii* uses specialized secretion systems to manipulate eukaryotic host cells through the injection of bacterial virulence proteins (effectors). However, current knowledge of how these pathogens establish infection is limited.

The central research goal of this work is to characterize changes in the transcriptome and proteome. Macrophages differentiated from the human monocyte THP-1 cell line were infected with two different strains (representatives of specific genomic groups) of *C. burnetii* in phase I, namely Scurry and Nine Mile. Samples were collected at days 3 and 7 post-infection (dpi) along with appropriate controls in three replicates and the intensity of infection associated with host cell viability was monitored. Subsequently, mRNA molecules and proteins were extracted. After sequencing, the data were further analyzed. The quality of transcript reads was checked using FastQC and mapped to the human reference genome (hg38 version) by Bowtie2. The limma (Linear Models for Microarray) add-on libraries of the R programming language and edgeR were used to determine differential gene expression, followed by gene set analysis (GSA – Gene Set Analysis). The bacterium was found to activate different signalling and metabolic pathways on the early (3rd dpi) and late (7th dpi) stages of infection in both isolates.

Also, proteins were subjected to systematic comparative MS/MS analysis. The final list showed in average of 2249 and 2195 identified proteins in the early and late infection, respectively. As many as 121 proteins were significantly altered in the early stage while 93 were changed in the late infection. The identified proteins were commonly cytoplasmic proteins with "moonlighting" activity. Large proportion of these proteins were responsible for interaction with the host's immune system. The results showed that the *C. burnetii* is able to reconfigure the intracellular environment to bypass or inhibit the host cell's defence functions. Proteins related to intercellular communication, known as "quorum sensing", have also been found. This could indicate that bacterial cells can coordinate their efforts in the host.

Keywords: macrophage, response, *C.burnetii*, transcriptomic, proteomics

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SURVIVAL STRATEGY OF THE TOLERANT *COXIELLA BURNETII* STRAIN TO DOXYCYCLINE EXPOSURE

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Abstract: *Coxiella burnetii* is a zoonotic intracellular pathogen that causes acute or chronic Q fever when infects humans. It is difficult to diagnose unambiguously since induces symptoms similar to common infections. This allows the emergence of outbreaks damaging the farming industry and human health (Fournier, P.E., J. Clin. Microbiol, 1998). Q-fever is treated with doxycycline, a highly cell membrane permeable antibiotic and one of the few that has proved some effectivity against *C. burnetii*. For chronic cases, the treatment using doxycycline can last several months on 100 µg daily doses due to the tolerance of the pathogen (Raoult,; D. Arch. Intern. Med, 1999). Understanding the mechanisms underlying this tolerance is crucial to implement effective therapies

To analyze the *C. burnetii* response, we compared the proteins obtained from cultures in axenic media antibiotic free and spiked with 0.5 µg/mL and 4.0 µg/mL of doxycycline. The proteins from the cellular pellet and the exhausted media were purified and digested with trypsin. The resulting peptides were separated by LC system Ultimate 3000 (Dionex) using a micro Pillar Array Column µPAC. MS detection was performed using Orbitrap Q-Exactive plus and a Waters Q-TOF Premier, identifying more than 800 and 162 proteins respectively. Found changes in the cell fraction were related to i) primary metabolism, ii) homeostasis of the cell wall, iii) cell division, and iv) oxidative stress detoxification. The soluble fraction showed changes in i) catalysis, ii) ribosome structure iii) transcription and iv) membrane structure. Samples of cells incubated in antibiotic were analyzed by Transmission Electron Microscopy. It was observed the cells changed from the Large Cell variant to the Small Variant

Given the change at protein level, the expression of key genes implicated with the TCA cycle (*glt*A), heat shock (*dna*K), membrane transport (*tol*B) and H_2O_2 scavenging (*ahp*C1) was analyzed showing increasing under antibiotic treatment. In a similar way, the amount of hydrogen peroxide showed a statistically significant increasing when the cells were doxycycline stressed. Other changes were the abundance of proteins involved in LPS synthesis, morphological modifications and the mechanisms against oxidative stress. Interestingly, we found that *C. burnetii* stays in a dormant state when cultured with antibiotic. With the obtained data, we developed a model to integrate the observed changes. We expect that understanding the mechanisms of protection that the bacteria use, will help to develop better treatments against *C. burnetii* infections.

Keywords: *Coxiella burneetii*, doxycycline, cell wall homeostasis

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THE PHOSPHORYLATION-DEPENDENT GENERATION OF HYPERFUSED MITOCHONDRIA DUE TO A NOVEL DRP1 MUTATION

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Abstract: Mitochondria is continuously in a state of fusion and fission to balance the mitochondrial morphology, dynamics, and function. Any imbalance in the mitochondrial proteins leads to pathologies. Drp1, a mitochondrial fission protein plays very crucial role in the proper division of mitochondria. It contains four domains GTPase, middle, variable and GED domains. The activity of Drp1 GTPase domain increases in many pathological conditions particularly in neurodegenerative disorders which causes excessive fission of the mitochondria and ultimately death of the neurons. The current investigation examines the role of a specific site of Drp1 protein. In this study we report a novel Drp1 mutation located in the GTPase domain of Drp1 protein (S39A) which upon overexpression showed a completely hyperfused mitochondria. We generated stably over-expressing Drp1 protein, Drp1 mutant protein (S39A) and mcherry positive cells using SHSY-5Y human neuroblastoma cell line. Mitochondrial morphology classification was done using High Content Screening. In addition to hyperfused mitochondria the Mutant over-expressed cells showed no hindrance in the production of ATP, proton leak and coupling efficiency as compare to control cells. This highlights the importance of Drp1 protein residue S39A located in the GTPase domain, for its outright activity in mitochondrial division. In addition, we speculate that this site is phosphorylated by an unknown kinase. Mass spectrometric analysis will confirm the kinase which will help us in determining the mitochondrial dysfunction due to phosphorylation at S39A. This study suggests the mutation at S39A results in clear alterations in Drp1 function and mitochondrial morphology that are likely involved in dynamic regulation of mitochondrial division in cells.

Keywords: Mitochondria, Drp1, GTPase domain, neurodegenerative disorders, mutation

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EFFECT OF LITHIUM ON MITOCHONDRIAL MORPHOLOGY AND BIOENERGETICS

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Abstract: Lithium is used since 1950's for the treatment of bipolar disorder. It has beneficial effect on the nervous system and stabilizes the mood changes. Lithium has a narrow therapeutic index and its concentration in serum should be kept in range of 0.4 to 1.2mM. The treatment with lithium is connected with low incidence of adverse effects on the cardiac function, which may lead to severe cardiac condition. As lithium is a monovalent cation, it is not surprising that it affects the sodium and potassium channels on the cardiac plasmalemma. Nevertheless, increased ROS level and altered metabolic activity (activity of dehydrogenases) were observed in isolated cardiomyocytes after up to 3 hours' incubation in therapeutic level of lithium. The adverse effects are reported in patients that have higher than therapeutic concentration of lithium in blood (1.5 - 3mM). Therefore, our aim was to determine the effect of the exposition to 2mM lithium during 48 hours on the cardiomyoblasts (h9c2 cell line) and their mitochondrial bioenergetics and morphology of mitochondrial network, which reflects the function of mitochondria. We measured the oxygen consumption rate of the intact cells. The maximal respiratory capacity comprises different types of oxygen consuming reactions: ATP-linked respiration, reserve respiratory capacity, proton leak and non-mitochondrial respiration. We found that the cells treated with lithium had compromised biophysical parameters of respiration: the cells exhibited significantly increased proton leak and the reserve respiratory capacity decreased to 80% of the control cells. These parameters were expressed relative to the maximal respiratory capacity. The maximal respiratory capacity itself was lowered in lithium-treated cells to 78±10% of control. The proportion of ATP-linked respiration, which reflects the cells' energetic needs, remained unaltered in both groups. The reserve respiratory capacity is mainly regulated by succinate dehydrogenase (complex II of the respiratory chain). Altered mitochondrial function is often reflected in the plasticity of the mitochondrial network. Therefore, we analysed the mitochondrial network morphology – number and length of branches, number of junctions, the total skeleton length per cell area and the number of isolated mitochondria (i.e. single branch with two ends). Though the mitochondrial network in the presence of lithium was unaltered in comparison to control cells, the unhealthy state of the mitochondrial energetics was indicated by the increased level of superoxide radicals in mitochondria. Our results indicate that the increased level of lithium can make the cardiac cells prone to failure, as the cells do not have the reserve to meet higher energetic demands. In combination with the reported interaction with ion channels on the plasmalemma, it can stand behind the severity of the adverse effects of lithium.

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Keywords: cardiomyoblasts, lithium, cellular respiration, mitochodnrial network

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INVESTIGATION OF NOVEL 4,7-DISUBSTITUTED COUMARIN DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

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Abstract: Owing to their intriguing physicochemical properties and a relatively simple and affordable synthesis, coumarin derivatives represent desirable heterocyclic scaffolds in the field of medicinal chemistry. Numerous coumarin derivatives with different substitution patterns have been isolated or synthetized since the first isolation of coumarin in 1820. These compounds exhibit a broad spectrum of biological activities including anticancer, antibacterial, antiviral, antifungal, antioxidant, neuroprotective, hepatoprotective and many other effects. Coumarin derivatives belong to low-molecular-weight ligands, the most attractive modern chemotherapeutics. The study of interactions between such ligands and their target biomacromolecules (nucleic acids, enzymes, other proteins) helps to elucidate their mechanism of action and biological activity profile. It has been proven that coumarin and especially its 7-hydroxy derivatives exhibit cytotoxic and antiproliferative activities against multiple human cancer cell lines. Such anticancer effects of small molecules often originate from their interaction with DNA and topoisomerases (Topo). The aim of our study was to elucidate the interaction mechanism of four newly synthetized 4,7-disubstituted coumarin derivatives **K1**-**K4** with ctDNA, monitor their inhibitory capacity against human Topo I and investigate their cytotoxic activity against A549 human lung carcinoma cell line. The results of spectroscopic studies (UV-Vis absorption spectroscopy, fluorescence emission spectroscopy, circular dichroism spectroscopy) and thermal denaturation studies confirmed a non-covalent interaction between the studied derivatives **K1**-**K4** and ctDNA. The compounds seem to preferably bind into the minor groove of duplex DNA through hydrogen bonds and van der Waals interactions. Topo I relaxation assay revealed that coumarin derivatives **K1**, **K3** and **K4** exhibit inhibitory activity against human Topo I, with compound **K1** being the most potent inhibitor from this series (inhibitory activity comparable with camptothecin). MTT assays were performed on A549 cancer cells after 24 and 48 hours of incubation with studied compounds **K1**-**K4** and unravelled that derivatives **K1** and **K2** displayed the most notable cytotoxic effect from the series, followed by the remaining compounds **K3** and **K4** that exhibited cytotoxicity to a considerably lower extent. Acknowledgement: This study was supported by VEGA Grant No. 1/00037/22.

Keywords: coumarin, DNA, topoisomerase I, A549, cancer

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IMPACT OF HRD1 INHIBITION ON SURVIVAL AND RESPONSE OF THE CELL LINES DERIVED FROM BRAIN TUMORS

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Abstract: Cancer and neurodegenerative diseases are the most serious problems of human medicine. Stress of endoplasmic reticulum (ER) is caused by the accumulation of misfolded proteins in the ER or in response to various conditions (hypoxia, oxidative stress, lack of growth signals, inadequate amino acid supplies, glucose deprivation and lactic acidosis). In tumour cells, ER stress is caused by their high degree of translation and metabolism as well as the tumour microenvironment. To respond to ER stress, eukaryotic cells have evolved a group of signal transduction pathways - the unfolded protein response (UPR). The UPR is controlled by the activation of three major receptors (sensors) in the ER membrane. These receptors are – inositol-requiring enzyme 1 (inositol-requiring enzyme 1, IRE1); PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6). UPR directs homeostasis restoration by arresting translation and increasing the expression of ER-specific chaperones and the activation of ER-associated degradation that involves retrotranslocation of misfolded proteins from the ER to the cytoplasm and their degradation by the proteasome. Retrotranslocation of misfolded proteins and their labelling with ubiquitin is supported by the E3 ubiquitin ligase HRD1 (HMG-CoA reductase degradation protein 1) in complex with SEL1L. Depending on the level of stress, apoptosis may also be initiated.

The aim of the project was to examine the impact of inhibition of HRD1 enzyme activity on the viability and molecular response of neuroblastoma SH-SY5Y as well as glioblastoma T98G and U87 cells.

We used specific inhibitor of HRD1 (LS-102). LS-102 selectively inhibits HRD1 enzymatic activity. This inhibitor suppress the proliferation of rheumatoid synovial cells and is a potential therapeutic target for rheumatoid arthritis.

We found that inhibition of HRD1 leads to the death of all investigated cell lines. Glioblastoma cells U87 exhibit the highest sensitivity to LS-102, while glioblastoma T98G cells were the most resistant to LS-102. We also observed significant increase of the HRD1 expression in T98G and U87 cells. Treatment of T98G cells with LS-102 leads to activation of the IRE1α-XBP1 pathway and consequent activation of IRE1α ribonuclease activity, that results in the splicing of XBP1 to XBP1s. Expression of HRD1 in neuroblastoma cells was also elevated but the change was not significant.

Our results indicate that the glioblastoma T98G and U87 cells compensate inhibition of HRD1 via its increased expression. Despite this, U87 cells exhibit the highest sensitivity to LS-102. We

assume that the increase in the expression of HRD1 as an essential protein of the ERAD process represents a certain compensatory mechanism by which cells try to cope with the inhibition of HRD1 activity.

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Keywords: endoplasmic reticulum, ER stress, ERAD, UPR

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RELATIONSHIP BETWEEN EXPRESSION OF MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITORS IN PATIENTS WITH BRAIN TUMOUR

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Abstract: Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) regulate processes associated with malignant behaviour. They contribute to cancer cells' invasiveness and migrating character by disrupting the basal membrane. Currently, the expression profile and role of various MMPs remain unclear. Moreover, only a few studies focus on differences in expression profiles of MMPs and TIMPs in glioblastoma (GBM) and meningioma (MNG). Using quantitative real-time PCR analysis, we identified the expression pattern of ECM modulators in GBM (n=20), astrocytoma (n=9) and MNG (n=16) biopsies. Subsequently, we also examined the protein levels of investigated MMPs and TIMPs by immunodetection. We found 5 deregulated genes in the malignant group compared to the benign meningioma group. Decreased expression was confirmed in MMP2, MMP3, MMP10 and TIMP2 genes, while elevated expression was observed only in TIMP4. Interestingly, we found that in almost all protein cases were the highest levels for glioblastomas in correlation with astrocytomas or meningiomas. In the pilot study, the association between the different expression of MMPs and TIMPs, and clinicopathological parameters reflects a role in predicting the aggressive behaviour of brain cancer.

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Keywords: brain tumours, metalloproteinases, qPCR, immunodetection

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COMPARATIVE ANALYSIS OF HUMAN MESENCHYMAL STEM CELLS DERIVED FROM VARIOUS SOURCES AND HUMAN FIBROBLASTS.

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Abstract: Mesenchymal stem cells (MSCs) hold tremendous potential in regenerative medicine due to their capacity for self-renewal, multipotent differentiation, minimal ethical restrictions, and immunomodulatory properties. However, MSCs derived from different human tissues exhibit variations in their differentiation potential and metabolic characteristics. These properties can significantly impact their therapeutic utility, particularly in tissue engineering. Although fibroblasts share similar cellular morphology and biological characteristics with MSCs, it remains unclear whether fibroblasts are functionally equivalent to MSCs for therapeutic purposes. This study aimed to compare various types of human MSCs and HDFa through cytological and molecular analysis. The differentiation potential of each MSC type and HDFa was assessed by evaluating their osteogenic, chondrogenic, and adipogenic differentiation over time, both in vitro and under 2D conditions. The morphological changes of the cells during each differentiation were monitored by light microscopy. The accumulation of lipid droplets, calcium deposits, and proteoglycans was analyzed and quantified using appropriate histochemical staining. Our results demonstrated various differentiation potentials among the studied MSC types and HDFa throughout the 21-day analysis period. DPSC cells exhibited a delayed yet more pronounced osteogenic capacity in the later stages compared to BMMSCs. As expected, ADMSCs exhibited excellent adipogenic differentiation potential, while DPSCs have shown no lipid droplet accumulation. Additionally, flow cytometry analysis revealed consistent expression of CD markers including CD73, CD34, CD90, and CD105. However, variations were observed in the expression of CD9. Furthermore, mitochondrial respiration, an essential component of cellular metabolism, was evaluated using O2k-FluoRespirometer. These findings highlight the importance of considering tissue source-specific characteristics when selecting human MSCs for regenerative medicine applications. Understanding the variations among MSC types will contribute to optimizing MSC-based therapies and facilitating their translation into clinical practice with a focus on personalized medicine.

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Keywords: mesenchymal stem cells, differentiation, mitochondrial respiration

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DO STRUCTURAL DIFFERENCES OF DECORIN BINDING PROTEINS FROM EUROPEAN *BORRELIA* GENOSPECIES INFLUENCE GLYCOSAMINOGLYCANS BINDING?

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Abstract: Adhesion of spirochetes from *Borrelia burgdorferi* sensu lato complex is the crucial step in the initial phase of Lyme disease infection. Decorin-binding proteins (Dbp) are glycosaminoglycan (GAG) binding adhesins exposed on the surface of Borrelia spirochetes. Dbps are expressed in two homologous forms, A and B, which were characterised as the main factors of Lyme Borrelia virulence. Based on the previously described differences in binding mechanisms of Dbp-GAG interaction, we focused on the relations between structural differences and GAG binding. We aim to describe the structural differences in detail among Dbps from European *Borrelia* species and their particular interactions with different GAGs using solution nuclear magnetic resonance (NMR) spectroscopy at atomic resolution. We achieved almost complete backbone and sidechain assignments of DbpA from *B. afzelii* and *B. bavariensi*s, and the assignment for other Dbp protein homologues is in progress. Secondary structure propensity based on chemical shifts and backbone dynamics (T_1 and T_2 relaxations, heteronuclear ^{1}H-^{15}N NOE) for both variants were compared with available NMR structures of North American *Borrelia* species. We also expressed selectively unlabelled proteins (KIT/ KILT amino acid combinations) for a more accurate assignment of methyl groups. We performed initial protein-GAG interaction studies of variants of DbpA/DbpB with different GAGs by NMR titrations and by hydrogen-deuterium exchange mass spectrometry (HDX-MS). Additionally, the Kd values of binding were measured by microscale thermophoresis. NMR-based structural and interaction analyses combined with HDX-MS experiments indicate species-specific differences in GAG binding and set the starting point for extensive detailed research of the influence of small structural and dynamic differences and their impact on GAG binding.

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KEY FACTORS AND CONSEQUENCES OF DRUG INDUCED GENOMIC CYTOSINE DEAMINATION

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Abstract: Many chemotherapeutic drugs perturb DNA integrity and/or impair DNA associated mechanisms. Uracil in DNA is one of the most frequent DNA modifications arising either by DNA deamination (spontaneous or enzymatic) or thymine replacing incorporation. This latter case is often exploited in treatment of colorectal cancer by using drugs which target the de novo thymidylate synthesis by inhibiting thymidylate synthase (TS) [1]. This way the dTTP precursor dTMP cannot be efficiently formed which can lead to the perturbation of the dUTP:dTTP ratio of the cellular pool resulting in frequent incorporation of uracil into the DNA during replication. Under such conditions uracil-DNA repair pathways might turn into hyperactivate futile cycles, leading to cell cycle arrest and finally cell death. However, in case of inhibited uracil-DNA repair, the resulted high genomic uracil content might also influence essential DNA regulatory processes ending in similar cell fates.

We have been studying the effects of two TS inhibitors widely used in cancer treatments: the base analogue 5-fluoro-2'-deoxyuridine (5FdUR) that forms stable complex with TS and methyl-tetrahydrofolate [2], and the TS selective antifolate, raltitrexed (RTX) [3]. Our previous U-DNA-Seq data from HCT116 cells with decreased uracil-DNA repair capacity showed that these drugs lead to somewhat different genomic uracil patterns despite inhibiting the same target enzyme [4]. We have also detected elevated frequency of genomic C-to-T transitions selectively in 5FdUR treated, repair deficient cells (unpublished). After thorough analysis of our data, we suspect that the C-to-T transitions are caused by APOBEC3 enzymes. One of our aims was to identify the APOBEC3 enzyme potentially responsible for the detected genomic deamination, therefore we measured their mRNA levels and we also investigated in the protein level and localization of APOBEC3s with increased gene expression levels. We also wanted to examine whether there are any phenotypic differences between the two TS inhibitors, so we checked the cell viability and the cell cycle upon treatment with different doses of the two drugs. For our surprise, we observed that the high dose of 5FdUR seems to be less effective than the low dose of 5FdUR or any dose of RTX, which also coincides with the elevated C-to-T mutation frequency detected exclusively upon high dose 5FdUR treatment. This may conclude that the mutations possibly caused by APOBEC3s might contribute to enhanced survival upon 5FdUR treatment. Identifying the molecular determinants of this behaviour in long-term might shed light on processes related to drug resistance frequently occurring during TS inhibitory anticancer treatments.

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Keywords: cytosine deamination, thymidylate synthase inhibitors, APOBEC3s

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PRODUCTION OF REVERSE TRANSCRIPTASE IN VIBRIO NATRIEGENS EXPRESSION SYSTEM

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Abstract: Reverse transcriptases are important enzymes of retroviruses that convert single-stranded RNA into double-stranded DNA. In molecular biology, they are used for quantitative RT-polymerase chain reaction, generation of cDNA libraries for cloning and many more. M-MuLV (Moloney Murine Leukemia Virus) reverse transcriptase contained proposed point mutations that provide high thermostability and processivity. *V. natriegens* is promising expression system for the production of recombinant proteins due to its rapid growth rate. With further advancements in genetic tools and optimization of cultivation strategies, *V. natriegens* has the potential to become a valuable alternative to established expression hosts, such *as Escherichia coli*, offering fast and efficient production of a wide range of recombinant proteins. The aim of our work was to produce M-MuLV reverse transcriptase using this expression host. We were able to transform *Vibrio natriegens* cells with construct of the pJexpress404 vector with M-MuLV reverse transcriptase and produce it intracellularly and to the cultivation medium, using overproduction of low molecular weight D,D-carboxypeptidases PBP5/6 enzymes that can affect the permeability of external cell structures and support the transport of proteins into the extracellular environment without the use of special secretion signals and additional permeabilization methods. In the next part of the work we will focus on optimization of production and determination of specific enzyme activity.

This research was supported by the Slovak Research and Development Agency grant APVV-21-0215.

Keywords: reverse trancriptase, *Vibrio natriegens*

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UNFOLDED PROTEIN RESPONSE IN MITOCHONDRIA AND ENDOPLASMIC RETICULUM OF SH-SY5Y CELLS INDUCED BY 3-NITROPROPIONIC ACID

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Abstract: In recent years, many researchers of malignant, neurodegenerative and cardiovascular diseases have been focusing on the study of mitochondrial or endoplasmic reticulum (ER) specific Unfolded Protein Response (UPR) and their mutual communication with an effort to identify molecular mechanism of the cause of mentioned diseases and potentially find the pharmacologic targets for treatment of these diseases. The disruption of homeostasis or dysfunction of either mitochondria or ER leads to the activation of organelle specific UPR, which cells use to restore homeostasis. Prolonged stress, when cells are not able to secure homeostasis, can lead to the cell death. It is known that mitochondria and ER have very close contact sites, which they use for communication and for exchange of Ca^{2+} ions, proteins, and lipids. Therefore, dysfunction of ER is associated with mitochondrial dysfunction and vice versa. In this study, we have used neuroblastoma cell line SH-SY5Y that is frequently used in neurobiology as an *in vitro* model for study of neurotoxicity in dopaminergic neurons. We treated our cells with 3-nitropropionic acid (3-NPA), which is irreversible inhibitor of complex II of respiratory chain. Relative viability of the cells was determined by MTT assay. We observed that relative viability of the cells treated with 1 mM 3-NPA for 48 and 72 hours was significantly decreased. We have also analysed expression of proteins that are markers of either ER or mitochondrial UPR using Western blot. We observed statistically significant changes in the levels of SEL1L, GRP78 and VDAC1 proteins in the cells treated with 3-NPA at 1 mM concentration for 24 hours and for GRP78 even at 0,2 mM concentration. Overexpression of SEL1L and GRP78 indicate that 3-NPA might induce ER stress. Although we did not observe changes in HRD1 protein level, which is involved in endoplasmic reticulum-associated degradation (ERAD), we suppose that ERAD is functional. Kaneko *et.al*. observed that upregulation of SEL1L did not precede that of HRD1. We suggest that ERAD was induced through ATF6 pathway but our hypothesis should be confirmed by further experiments. In addition, the upregulation of VDAC1, protein which is part of mitochondria associated membranes (MAMs), indicate that there is a cross communication between mitochondria and ER and that stress of mitochondria, could potentially trigger the ER stress response.
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Keywords: endoplasmic reticulum, mitochondria, 3-nitropropionic acid, unfolded protein response

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INITIAL STEPS IN THE STUDY OF CATALASE FUNCTION AND LOCALISATION IN *LEPTOMONAS SEYMOURI*

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Abstract: Catalase is a ubiquitous enzyme involved in the protection against reactive oxygen species (ROS). Its main function is the decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is not only a harmful molecule, it is also known to participate in redox signalling and regulation of biological activities by the oxidation of thiolate groups. Therefore, it is important to keep the level of this molecule in the nM range in a cell. This task is accomplished by a set of different enzymes (peroxidases, catalase, peroxiredoxins).

Despite its wide distribution, some eukaryotic lineages lack catalase. The catalase-encoding gene is, for instance, absent in species inhabiting anoxic conditions, parasitizing in blood, or photosynthetic eukaryotes with secondary plastids. An interesting pattern of catalase distribution can be found in the family Trypanosomatidae. It has been shown that the gene encoding catalase was acquired three times independently from three different bacterial lineages *via* horizontal gene transfer by the monoxenous Leishmaniinae (from *Brachyspira* spp.), Blastocrithidiinae (from *Snodgrassella* spp.), and *Vickermania* spp. (from *Acinetobacter* spp.). Subfamily Leishmaniinae is an intriguing example to study catalases of Trypanosomatidae. It unites dixenous (with two hosts in the life cycle), medically important species (*Leishmania sensu lato*) and monoxenous (one host in the life cycle) species. The catalase gene is present in the genomes of monoxenous relatives and was secondarily lost in dixenous species suggesting that presence of this enzyme is incompatible with dixeny.

Here we investigated the role of catalase in *Leptomonas seymouri*, a monoxenous trypanosomatid of the subfamily Leishmaniinae. This species is thermotolerant and was often documented in immunocompromised patients or co-infections with *Leishmania donovani.*

We report that *L. seymouri* is amenable to genetic manipulations using conventional and CRISPR/Cas9-mediated approaches by establishing lines with catalase ablation (KO) and add-back (AB). Three investigated cell lines (WT, KO, and AB) were similar in the growth kinetics and morphology at both 23 and 35°C, while the cytotoxicity assay revealed an increased resistance to hydrogen peroxide in case of AB compared to WT and KO counterparts (EC_{50} at 23°C: 1,76; 1,73; 4,07 mM H_2O_2, respectively) and decreased resistance of KO in comparison to WT at 35°C (EC_{50} at 35°C: 3,57; 2,72; 6,75 mM H_2O_2, respectively. The cell line with endogenously tagged catalase was used to study its localization (by IFA and biochemically). We demonstrated that this enzyme has dual localization – glycosomes and cytoplasm – unifying previously reported contradicting results in trypanosomatids. Moreover, the proportion of cytoplasmic catalase increased significantly upon treatment with hydrogen peroxide.

Keywords: Leptomonas seymouri, trypanosomatids, catalase

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COMPARISON OF THE EFFECT OF ALIPHATIC AND AROMATIC ISOTHIOCYANATES ON LEUKEMIC CELLS.

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Abstract: Isothiocyanates (ITCs) are a group of sulphur-containing aliphatic and aromatic substances that occur in nature (sulforaphane, allyl isothiocyanate, benzyl isothiocyanate, phenethyl isothiocyanate, etc.) or as chemical synthetic products (fluorescein isothiocyanate, p-bromophenyl isothiocyanate, etc.) and have a wide range of biological effects. They are the products of hydrolysis of glucosinolates by endogenous plant myrosinase enzymes found in various vegetables (cauliflower, broccoli, cabbage, mustard, horseradish, wasabi). This hydrolysis takes place when the tissues are mechanically damaged by pests. The effects of ITC can be derived from the reactivity of the ITC group (-NCS) and the physicochemical properties (lipophilicity, shape, size and stiffness) of the residue molecule. While the former predicts the ability of ITCs to react with functional groups of either small biomolecules or biopolymers, the latter is responsible for their bioavailability in different compartments of cells and tissues. NCS groups are amenable to attack by nucleophilic functional groups with a free electron pair and a partial negative charge. In biological materials, the most common reactive partners of ITCs are the -SH, -OH and -NH2 groups. The biological effects of ITCs resulting from their chemical properties have been intensively studied in the last four decades of the last century. These compounds have been reported to have significant biological effects including anticancer, anti-inflammatory and antimicrobial activities. In our work, we studied two structurally distinct ITCs: sulforaphane (SFN) and benzyl isothiocyanate (BITC). We investigated the cytotoxic activity of these two compounds on parental human leukemia cells (SKM-1 and MOLM-13) as well as on P-gp positive sublines of these cells (SKM/VCR and MOLM/VCR). We showed that BITC had a stronger inhibitory effect on cell proliferation. We also found that P-gp phenotype may or may not modulate the antiproliferative effects of ITCs. Compared with BITC, SFN inhibited cell metabolic activity at lower concentrations. Cell death assay demonstrated the ability of ITC to induce an apoptotic mode of cell death, whereas BITC was more effective. The P-gp phenotype, which enhanced cell resistance to ITC, also played an important role in the different sensitivity of cells in inducing apoptosis. The differences in the effect of aliphatic and aromatic ITCs are due to their different physicochemical properties.
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Keywords: benzyl isothiocyanate; sulforaphane; apoptosis; isothiocyanates

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IN SILICO INVESTIGATION OF THE POTENTIAL MECHANISM OF THE PDZ INTERACTION BETWEEN GAT1 AND SYNTENIN-1

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Abstract: The major inhibitory neurotransmitter in the mammalian central nervous system, gamma-aminobutyric acid (GABA), has transporters and receptors that work in GABA signaling regulation connected with normal brain development and homeostasis. The GABA transporter GAT1 belongs to the Na^+/Cl^--dependent family of neurotransmitter transporters, and it has 12 transmembrane domains with N- and C-terminal regions in the cytoplasmic site [1, 2]. The C-terminus of GAT1 (-E**AYI)** contains a PDZ binding motif recognizable by proteins with PDZ domains. This PDZ binding motif belongs to class II, Φ-X-Φ, where the crucial hydrophobic amino acid is Φ in PDZ positions 0 and -2, and X is whichever flexible amino acid in position -1 [3,4].

Previously we show that GAT1 directly interacts with syntenin-1, exactly C-terminal PDZ binding motif interacts mainly with syntenin-1 PDZ domain 1 through the last two amino acids of the PDZ binding motif, isoleucine 599 in the position 0 and tyrosine 598 in the position -1. It indicates the existence of an unconventional PDZ interaction mode, where tyrosine 598 can be potentially phosphorylated, and so it could cause regulation of this PDZ interaction [5].

In this work, we focused on *in silico* investigation of the possible mechanism of the PDZ interaction between the GAT1 PDZ binding motif and syntenin-1 PDZ domain 1. We used Autodock Vina [6], and then the structures were further analysed using PyMOL (The PyMOL Molecular Graphics System, Schrodinger, LLC; http://www.pymol.org). From our *in silico* results, isoleucine 599 in position 0 is in a conserved hydrophobic carboxylate loop. This assumption supports the fact that the PDZ interaction is lost when terminal hydrophobic isoleucine is replaced by polar serine. The tyrosine 598 likely interacts with lysine 125 via Π cationic interactions and directs its OH group against the negative aspartic acid doublet (D121, D123). In the case of tyrosine phosphorylation, this will likely cause significant repulsion of the negatively charged phosphorylated residue and prevent the interaction.

Keywords: : GABA transporter GAT1, syntenin-1, PDZ interaction
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CELL DEATH INDUCED BY HYPOMETHYLATING AGENTS IN MOLM-13 CELLS

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Abstract: In the treatment of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), two commonly used hypomethylating agents (HMAs) are 5-azacytidine (AZA) and 5-aza-2'-deoxycytidine (DAC). These agents are predominantly used in older patients who are unsuitable for intensive chemotherapy and hematopoietic stem cell transplantation. However, only 30-60% of patients with MDS and 18-47% of patients with AML respond to this therapy. We established two variants of the MOLM-13 human acute myeloid leukemia cell line to investigate drug resistance. The first variant, MOLM-13/DAC, exhibited resistance to DAC, while the second variant, MOLM-13/AZA, showed resistance to AZA. To obtain these variants, we subjected the cells to a six-month selection/adaptation process with gradually increasing concentrations of either DAC or AZA. MOLM-13/DAC cells displayed resistance to DAC (approximately 50-fold), while MOLM-13/AZA cells demonstrated resistance to AZA (approximately 20-fold). Notably, we did not observe cross-resistance between MOLM-13/DAC and AZA, or between MOLM-13/AZA and DAC. To investigate the mode of cell death, we evaluated the retention of fluorescein-linked annexin V and propidium iodide in the cells. Our findings revealed an apoptotic cell death mechanism in MOLM-13 cells following treatment with DAC or AZA, in MOLM-13/DAC cells following treatment with AZA, and in MOLM-13/AZA cells following treatment with DAC. Through the JC-1 assay, we also observed a decrease in mitochondrial membrane potential as the cells progressed towards apoptosis. Additionally, we examined the methylation levels of promoter regions in genes encoding apoptosis-regulating proteins and studied the relationship between this methylation and the expression of the respective genes. Furthermore, we focused on determining the expression levels and activity of proteins involved in both the intrinsic and extrinsic apoptosis pathways. Upon treatment with hypomethylating agents, the cells underwent apoptosis as the mode of cell death. In the parental MOLM-13 cells, the extrinsic apoptosis pathway was activated following treatment with both HMAs. In contrast, AZA treatment in MOLM-13/DAC cells and DAC treatment in MOLM-13/AZA cells induced apoptosis through a combination of the extrinsic and intrinsic pathways. Each cell variant serves as a suitable model for investigating and characterizing the differential distribution of resistance between the two HMAs.
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Keywords: 5-azacytidine; 5-aza-2′-deoxycytidine; acute myeloid leukemia, myelodysplastic syndromes; chemoresistance

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ACTIVATION OF A SILENT BIOSYNTHETIC GENE CLUSTER FOR A TYPE I POLYKETIDE SYNTHASE SECONDARY METABOLITE IN *STREPTOMYCES LAVENDULAE* SUBSP. *LAVENDULAE* CCM 3239

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Abstract: The Gram-positive bacteria of the genus *Streptomyces* represent the main producers of secondary metabolites. Under laboratory conditions, one strain of *Streptomyces* produces one or several secondary metabolites with a broad range of biological activities, including antibiotics, anticancer, antifungals, antivirals, etc. Bioinformatic analyzes of the complete genome sequence of our model strain *S. lavendulae* subsp. *lavendulae* CCM 3239 revealed 30 silent biosynthetic gene clusters (BGCs) that encode natural product pathways. Seven of these thirty BGCs encode potential polyketides; two of them represent putative type I PKS. Type I PKS are large modular multifunctional proteins that elongate, process, and terminate the polyketide chain in a unique manner in one single condensation cycle. Each module contains a set of distinct acyltransferase, ketosynthase, and acyl carrier protein catalytic domains that work together to form a β-ketoester intermediate. Other modules such as ketoreductase, dehydratase, and enoylreductase are responsible for the modification of the keto group. In the process of polyketide formation, the growing polyketide chain is transferred from one module to another until the complete molecule is released from the last module by a thioesterase. Type I PKSs are responsible for the production of reduced polyketides such as macrolides, polyethers and polyenes. The aim of this work is to establish the activation strategy of selected unknown BGCs by integrating the strong *kasOp** promoter in front of the genes of positive regulators or biosynthetic genes into the *Streptomyces* chromosome. To validate the strategy, we selected a silent BGC2 for type I PKS by inserting a strong *kasOp** promoter upstream the *SLAV_02840* gene encoding a LAL family transcriptional activator and *SLAV_02776* gene encoding a type I PKS biosynthetic gene in *S. lavendulae* subsp. *lavendulae* CCM 3239. DNA fragments surrounding the promoter region of both genes amplified by PCR and cloned into a vector that was used to integrate the *kasOp** promoter into the chromosome by homologous recombination. Phenotypic analysis of the mutant *S. lavendulae*, *kasOp::2840* strains compared to WT revealed a dramatic overproduction of yellow compound inhibiting growth of yeast, corresponding to the polyene Strevertene A. Interestingly, the *S. lavendulae*, *kasOp::2776* mutant strains overproduced a similar yellow compound inhibiting yeast growth that differed from Strevertene A. These results confirmed the efficient activation of the silent BGC2 for type I PKS using this system.
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Keywords: antibiotics, polyketides, strevertenes, *Streptomyces*

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ENGINEERING OF A NOVEL TWO-ENZYME FUSION SYSTEM FOR PET DEGRADATION

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Abstract: Polyethylene terephthalate (PET) is one of the most abundantly used polyester, with annual production exceeding 50 million tons. PET waste accumulated in landfills is predicted to persist for centuries to millennia. Its fragmentation generates microplastics (MPs), which have been found in both water and soil, and more recently have been observed entrained in the air or in marine animals. Therefore MPs are presumed a serious threat to various organisms and human health.
Biodegradation of plastic pollution offers environmentally friendly route for waste recycling. Enzymatic breakdown of PET has been studied extensively with various PET-degrading enzymes identified. PET hydrolase (PETase) secreted by *Ideonella sakaiensis* converts PET with a relatively high activity. A number of PETase variants have been designed to improve protein stability and increase its activity, however the enzymatic properties remain insufficient for the industrial application. Further studies revealed that dual-enzyme systems synergy increased PET depolymerization, which was further enhanced by linking the enzymes.
The aim of our project is to contribute to the development and evaluation of a novel highly effective two-enzyme fusion system capable to hydrolyze PET to monomers important for subsequent reuse in new products, thereby contributing towards the concept of a circular PET economy.

Keywords: polyethylene terephthalate (PET); biodegradation; PET-degrading enzymes; PETase; protein engineering; chimeric protein

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AVIAN ORTHOREOVIRUS σNS STRUCTURE AND FUNCTION

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Abstract: Fusogenic avian orthoreoviruses of the family Reoviridae are important avian pathogens that can cause significant economic losses in the poultry industry and are associated with a variety of poultry diseases and exhibit icosahedral, non-enveloped virions and 10 dsRNA genomic segments (23.5 kb)[1,2]. RNA replication and reovirus morphogenesis occur exclusively in cytoplasmic inclusion bodies, also known as viral factories or viroplasms. Viroplasms are formed by the non-structural protein μNS in association with the non-structural protein σNS[1]. Each progeny of avian reovirus (ARV) must encapsidate a complete set of 10 different genomic segments. However, due to the low probability of selecting the complete genome by a random assortment, a specific RNA assembly mechanism is required. The RNA chaperone activity of the σNS protein, whose oligomeric state dynamically controls its activity, is thought to be responsible for this process through a series of sequence-specific RNA-RNA interactions[3,4]. In order to elucidate the molecular mechanism we have imaged σNS apoprotein using cryo-EM, after many unsuccessful attempts with X-ray crystallography, and reconstructed an electron density map and build an atomic model for octamers which are further stabilized by RNA binding. In addition, long RNAs induced the assembly of filamentous ribonucleoproteins (RNPs). Using a deletion mutant, we show that the N-terminal tail of σNS is required for filament and octamer formation. Structural analysis of the octameric σNS provides basis for mechanism of assembly of the dimeric building block as well as higher oligomeric species and RNPs.

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Keywords: Avian orthoreovirus, sigmaNS, cryoEM, viral factories, RNA chaperone

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GENOMIC INSIGHT INTO ADAPTATION OF ACINETOBACTERS TO EXTREME ENVIRONMENTS

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Abstract: Extreme environmental conditions (e.g., very high/low temperature, pH, humidity, salinity, pressure, lack of nutrients, presence of toxic substances, etc.) significantly influence biodiversity. Generally, the extreme conditions lead to a reduction in the diversity of organisms; on the other hand, these environments can be a source of unique organisms with a high biotechnological potential. Microorganisms inhabiting extreme environments have developed various adaptation mechanisms allowing them to survive in inhospitable conditions. The structure of the genome and its changes (the acquisition or loss of some genes through natural selection, genetic recombination, mutations or horizontal gene transfer) play a significant role in microbial adaptation processes. In this study, the whole-genome sequence analysis of two *Acinetobacter* strains was performed to identify genetic determinants associated with their adaptation to heavy-metal polluted environments. The strain RB2-047 was identified in gold-bearing ore obtained from the Rozália Gold Mine in Hodruša-Hámre village; the strain K1 originated from a brown mud created during aluminum production near the city Žiar nad Hronom. The bioinformatic analyses applied showed that the ability of the *Acinetobacter* strain RB2-047 to survive in high heavy-metal polluted mine environment is mediated by the presence of numerous multidrug efflux pumps encoded by chromosomally located genes (*bcr*, *pATP*, *tolC*, *corC*, *ydhE/norM*, *chrAB*). There were also found copper resistance genes *copBCD* and transcriptional regulators *cusSR*, *merR* and *cadR* in the RB2-047 genome. Three contigs were identified as plasmid sequences, however, they do not carry any genetic determinants of metal resistance. The *Acinetobacter* strain K1 show several chromosomally and plasmid located genes associated with the adaptation to environmental conditions of brown mud (e.g., efflux pump genes *czcA*, *czcD*, *tolC*, *arcA*, *cadR-pbrR*, *zntA, zitB;* or metal and metaloid resistance genes *copABCDZ*, *cusSR*, *arsHCOR*, *acr3*). The most heavy-metal resistance encoding operons are chromosomally located and at least 10 contigs identified as plasmid sequences carry metal or metalloid resistance genes. Our results indicate that the efflux activity may be the main adaptation mechanism in isolated bacterial communities (as in gold mine) and genetic determinants are mainly chromosomally located. Less isolated bacterial communities (as in brown mud) may contain higher number of plasmids carrying various efflux pump and metal-resistance genes with the possibility of their horizontal transfer within bacterial community.

Keywords: *Acinetobacter* spp., heavy metal, extreme environment, gold mine, brown mud

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DIFFERENTIAL *IN VIVO* RECOGNITION OF PROMOTERS RECOGNIZED BY NINE SIGB HOMOLOGUES PRESENT IN *STREPTOMYCES COELICOLOR* A3(2)

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Abstract: In their natural environment, bacteria are exposed to various stresses. The ability to modulate gene expression in response to these stresses is mediated in many bacteria by stress-response sigma factors of RNA polymerase that control expression of genes encoding stress proteins necessary to overcome these adverse conditions. The stress-response sigma factor SigB of Gram-positive *Bacillus subtilis* is the best-characterized example. Its activity is regulated by the RsbW/RsbV partner-switching mechanism. Under non-stress conditions, SigB is sequestered by the anti-sigma factor RsbW. The release of the sigma factor from this complex is accomplished by the anti-anti-sigma factor RsbV, which is dephosphorylated by specific PP2C-type phosphatases RsbU/RsbP under stress conditions and sequesters RsbW. In addition, RsbW specifically phosphorylates RsbV through the serine protein kinase activity of its HATPase_c domain, providing negative feedback for SigB activation. Unlike *B. subtilis*, the Gram-positive soil bacterium *Streptomyces coelicolor* A3(2), which undergoes a complex cycle of morphological differentiation, contains nine SigB homologues (SigBFGHIKLMN) with a major role in differentiation and osmotic stress response. In addition, it contains 45 RsbW homologues, 17 RsbV homologues, and 44 RsbU/RsbP homologues, suggesting a rather complex regulation of these SigB homologues compared to *B. subtilis*. We previously constructed a heterologous two-plasmid *E. coli* system to identify promoters recognized by sigma factors and used it to identify promoters recognized by these nine SigB homologues. Interestingly, almost all identified promoters were recognized by two or more SigB homologues. However, promoter analysis did not reveal any specific sequences characteristic for these recognition groups. All promoters showed high similarity in the −35 and −10 regions. To examine this promoter cross-recognition *in vivo* in *S. coelicolor* A3 (2), several promoters were inserted in a luciferase reporter plasmid and conjugated to a wild-type strain of *S. coelicolor* A3(2) and nine mutant strains of *S. coelicolor* containing deleted individual sigma factor genes. Luciferase reporter activity indicated differential activity of these promoters in these mutant strains, suggesting overlapping promoter recognition by these nine SigB homologues in *S. coelicolor* A3(2).

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Keywords: differentiation, promoter, sigma factor, Streptomyces

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INFLUENCE OF QUERCETIN AND GLYCEMIA ON THE EXPRESSION OF ACE2 AND Na,K-ATPase IN ZDF RATS

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Introduction: Diabetic nephropathy is considered a severe complication of diabetes. The aim of this study was to investigate the influence of hyperglycemia on transmembrane enzymes involved in maintaining osmotic balance, namely Na,K-ATPase (NKA) and angiotensin-converting enzyme (ACE)-2. Additionally, we were interested in the impact of administering the flavonoid quercetin (QCT) on the renal expression of these enzymes in diabetic animals.
Methods: We used 6-month-old obese Zucker diabetic fatty (ZDF) rats as the model of Diabetes type 2 and included lean ZDF rats as the control group. QCT (20 mg/kg/day) was orally administered to the rats for 6 weeks. *In vitro* activation of renal NKA was performed using either Na+ (2-100 mmol/L) or ATP (0.16-8.0 mmol/L) to determine the maximum enzymatic reaction rate (Vmax) and characterize the enzyme's ability to bind ATP and Na+ ions (Km, KNa). Western blot analysis was employed to measure the expression of ACE2, as well as the individual isoforms of NKA (α1, β1).
Results: We observed that the ZDF model exhibited varying levels of glycemia in diabetic rats, prompting us to further divide the groups into those with lower hyperglycemia (Glc <11 mmol/L; D low) and higher hyperglycemia (Glc>11 mmol/L; D high). The same approach was applied to the diabetic group receiving QCT (DQ low and DQ high). Based on this division, we found that as the glucose level increased in obese ZDF rats, the Vmax of renal NKA also increased. Administration of QCT reduced NKA activity in control animals, particularly in the DQ low diabetic rats. However, the affinity for Na+ ions or ATP was not significantly affected. By Western blot method, we observed significantly higher expression of NKA subunits (α1 and β1) in the D low group compared to the control, with no significant effect on ACE2 expression. Furthermore, higher protein expression levels of NKA and ACE2 were found in the DQ high group compared to DQ low. Conversely, markedly lower expression of NKA subunits and ACE2 was observed in the diabetic D high group compared to the D low group. Interestingly, administration of QCT to the DQ high group resulted in increased expression of the investigated proteins (ACE2, NKA) compared to the D high group.
Conclusion: Our findings suggest that higher NKA activity partially correlates with increasing glycemia in ZDF rats, which could be associated with a higher rate of sodium reabsorption into the blood. Kidney damage in type 2 diabetes is also linked to changes in protein expression, as indicated by significantly lower ACE2 expression. Administration of QCT to obese diabetic rats with hyperglycemia (Glc <11 mmol/L) partially reduced NKA activity but mainly affected the expression of ACE2 and NKA. On the other hand, administration of QCT to obese diabetic rats with hyperglycemia (Glc>11 mmol/L) increased the expression of both enzymes, ACE2 and NKA.
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CHANGES IN HUMAN NEURAL CELLS AFTER APPLICATION OF STATINS

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Abstract: Statins have an important place in medicine due to their high success rate in the treatment of cardiovascular diseases. For this reason, they are currently the most frequently prescribed drugs for the regulation of cardiovascular diseases. The main effect of statins is the inhibition of the conversion of β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate. This result leads to the inhibition of sterol production. The most important effect is the inhibition of cholesterol production because this molecule is an essential part of cell membranes. The result of this process can be a decrease in membrane integrity connected to the destruction of cancer cells. Another significant effect of statins is the inhibition of isoprenylation of key oncoproteins involved in the regulation of proliferation and the cell cycle [1]. Pleiotropic effects of statins include the suppression of growth, survival, migration of tumor cells, formation of metastasis, angiogenesis, inflammation, as well as support of cell death - apoptosis [2].
Recent research has shown the positive effect of statins in the treatment of various cancer diseases such as hepatocellular carcinoma, lung cancer, colorectal cancer, and prostate cancer. Nowadays, scientific knowledge about the effect of statins on brain tumors and healthy cells is insufficient or diverse. One of the most malignant types of brain tumors is glioblastoma. This type of tumor is associated with high mortality, and the median overall survival of patients with glioblastoma is very low, approximately 15 months from the onset of the disease (after surgical resection of the tumor, radiotherapy, or chemotherapy). Our aim is to clarify the molecular mechanisms induced by the presence of statins in tumor brain tissue and point out the possible adjuvant effect of statins during brain cancer treatment.
In our project, we focused on comparing the impact of simvastatin on healthy astrocytes and cancerous glioblastoma cells. We monitored the confluence and morphology of cells in the presence of simvastatin. We also studied the effect of simvastatin on cell viability in a time- and concentration-dependent manner. Moreover, we prepared 3D cell spheroids to analyze the more complex cellular processes. We found out that simvastatin had a supportive effect on healthy cells at very low concentrations but a strong inhibitory impact on cell viability at higher concentrations. Healthy cells are much more sensitive to increased concentrations of simvastatin compared to cancer cells.
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BENEFIT OF NGS ANALYSIS FOR TARGETED THERAPY OF LUNG ADENOCARCINOMA

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Abstract: Lung cancer belongs to the leading causes of cancer deaths worldwide. Based on histology, lung cancer is categorized into small cell lung cancer and non-small cell lung cancer (NSCLC). Adenocarcinoma is the most common subtype of NSCLC. Targeted therapy has become increasingly important in treating lung adenocarcinoma. Next-generation sequencing (NGS) enables precise identification of specific genetic alterations in individual tumour tissues, thereby guiding targeted therapy selection. This study aimed to analyze mutations present in adenocarcinoma tissues using NGS, assess the benefit of targeted therapy and evaluate the progress in availability of targeted therapies over last five years. The study included 237 lung adenocarcinoma patients treated in 2018-2020 at University Hospital in Pilsen. The Archer FusionPlex CTL panel was used for NGS analysis. Gene variants covered by the panel were detected in 57 % patients and fusion genes in 6 % patients. At the time of the study, 34 patients (14.3 % of patients) were identified with a targetable variant. Twenty-five patients with EGFR variants, 8 patients with EML4-ALK fusion and one patient with CD74-ROS1 fusion received targeted therapy. Prognosis of patients at advanced stages with EGFR variants treated by tyrosine kinase inhibitors and patients with EML4-ALK fusion treated by alectinib was significantly favourable compared to patients without any targetable variant treated by chemotherapy (p=0.0172, p=0.0096, respectively). Based on treatment guidelines applicable in May 2023, the number of patients who could profit from targeted therapy would be 64 (27.0 % of patients), this is an increase by 88 % in comparison to recommendations valid in 2018-2020. Due to the expanding spectrum of targetable mutations, more and more patients could profit from the assessment of mutational profiles using NGS which could become a crucial approach in the routine management of oncological patients. *The work was supported Conceptual Development of Research Organization (Faculty Hospital in Pilsen-FNPl, 00669806), by the Cooperatio Program, and by the project of Faculty of Medicine in Pilsen SVV-2023-260654.*

Keywords: lung adenocarcinoma, next generation sequencing, targeted therapy, prognosis

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HYPERHOMOCYSTEINEMIA AS RISK FACTOR FOR ISCHEMIC STROKE AND NEURODEGENERATION

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Abstract: Homocysteine (Hcy), an intermediate product of methionine (Met) metabolism, is a sulfur-containing non-proteinogenic amino acid whose physiologic level in plasma is determined by dietary intake, dysregulation of the Met-Hcy metabolism and low vitamin B status. Hyperhomocysteinemia (hHcy) is responsible for developing cardio- and cerebrovascular diseases, including ischemic stroke. We provide overview of the hHCy effect on plasma metabolome, histomorphological and biochemical alterations of brain parenchyma. It is known that hHcy-induces oxidative stress, inflammation and endoplasmic reticulum (ER) stress, considered to play an essential role in the pathogenesis of several neurodegenerative diseases. Hippocampal neurons are sensitive to the prolonged level of Hcy due to absence of metabolization by the transsulfuration as well as by folate or by B12 independent remethylation. Study also highlights an active role of hHcy in the pathological changes, such as amyloid deposition, the occurrence of hyperphosphorylation of tau protein, which exacerbates if combined with global ischemia-reperfusion injury (IRI). Experimentally induced hHcy by Met diet alters plasma and hippocampal metabolome, behavioural and histo-morphological patterns in rats, due to changes in "methylation index", which eventually aggravates the noxious effect of high methionine intake. We provide a survey into the molecular mechanisms of how hHcy itself, or with combination with IRI, could endorse neurodegeneration. Prevention of risk factors such as hHcy, ischemic stroke, appear to have important implications for development of neurodegeneration and deserves investigation.

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Keywords: hyperhomocysteinemia, ischemia, neurodegeneration**,** metabolome,

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PRODUCTION OF DEK1 LG3 DOMAIN IN VIBRIO NATRIEGENS

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Abstract: The production of recombinant proteins is one of the most important and developing areas in the field of molecular biology. The need to choose suitable conditions for production, such as the selection of suitable hosts, editing of expression vectors and, last but not least, financial requirements, is the most important step in the construction of large-capacity expression systems. *Vibrio natriegens* is considered to be the fastest growing freeliving bacterium with a culture doubling time between 7 and 10 minutes under optimal conditions. Estimates of the number of ribosomes in *V. natriegens* are approximately 115,000 per cell in the exponential phase of growth, while in *E. coli* the number is estimated to be only 70,000–90,000, resulting in a higher rate of biomass synthesis and a stronger ability to synthesize proteins. In this study, we focus on the production of the LG3 domain from the plant calpain DEK1 precisely in *V. natriegens* strains, which can help in better production and purification of the produced domain.

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Keywords: *Vibtio natriegens*, *DEK1*, *LG3 domain, heterologous expression*

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PRODUCTION OF DEK1 PROTEIN FRAGMENTS IN ESCHERICHIA COLI

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Abstract:
Calpains are cysteine proteases responsible for the regulation of several cellular processes. The only known calpain in plants is DEFECTIVE KERNEL 1 (DEK1). It is a membrane protein containing calpain protease at its C-terminus. Its correct function is essential for the correct development of the aleurone layer of cells in the endosperm of plant seeds, embryogenesis or complex three-dimensional plant growth. It plays an important role in the growth and development of the plant, but despite this, the molecular mechanism of its functioning is not exactly clarified. In this work, we focus on the optimization of the production of plant DEK1 calpain domains in the *Escherichia coli* expression system. Optimizing the production process also includes designing a suitable combination of purification methods in order to achieve the highest possible yield with sufficient purity for pre-crystallization studies. Despite several optimizations of the entire production and purification procedure, we managed to achieve only partial purity of individual domains of DEK1 calpain, as protein aggregation occurred during the process.

This research was supported by the Slovak Research and Development Agency grant APVV-21-0227 and by the projects UpScale of Comenius University Capacities and Competence in Research, Development and Innovation, ITMS: 313021BUZ3 and NUKLEUS 313011V387, co-financed by the European Regional Development Fund within the Operational Programme Integrated Infrastructure.

Keywords: *Escherichia coli*, *DEK1*, *LG3 domain, heterologous expression*

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THE EFFECT OF TUDCA AND PBA ON MITOCHONDRIA IN SH-SY5Y CELLS

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Abstract: Mitochondria are the major site of ATP synthesis in eukaryotic cells. In addition, they participate in various other biological processes, including apoptosis, cell senescence, autophagy, or calcium homeostasis. These highly dynamic organelles are particularly essential for neurones because of their high-energy demands. Improvement in mitochondrial activity may contribute to prevention of neurodegeneration, offering a new therapeutic opportunity for neurodegenerative diseases.

Tauroursodeoxycholic acid (TUDCA) and 4-phenylbutyric acid (PBA) are approved by the Food and Drug Administration (FDA) for the treatment of biliary cirrhosis and urea cycle disorders. TUDCA is a hydrophilic bile acid naturally produced by conjugation with taurine in the liver. Short-chain fatty acid, PBA, is a derivative of butyric acid produced by fermentation of colonic bacteria. These blood-brain barrier penetrating substances appear to be anti-apoptotic molecules with chaperone-like activities, but the precise mode of the action still remains elusive.

In this study, we have investigated the effect of TUDCA and PBA on mitochondrial functions, such as mitochondrial respiration, generation of mitochondrial membrane potential, ATP synthesis capacity, as well as mitochondrial morphology in human neuroblastoma cells SH-SY5Y that are often used in neurobiology as an *in vitro* model for study of neurodegeneration of dopaminergic neurons. TUDCA does not exhibit a significant impact on the mitochondrial level and also morphology in SH-SY5Y cells. Although basal respiration, electron transfer (ET) capacity, succinate-driven respiration, and ATP-coupled respiration were decreased, functional properties, including ATP levels and membrane potential, were preserved. In contrast, PBA significantly increased ET capacity and spare respiratory capacity (SRC). PBA also induces significant changes in generation of mitochondrial membrane potential, in particular it decreases an extent of depolarisation of mitochondrial potential after addition of ADP, that could be a result of increased SRC. In addition, PBA could increase the measure of mitochondrial fusion.

In conclusion, the results presented in this study suggest that treatment of cells with PBA might increase cell resistance against different stress conditions that could be attributed to the impact of PBA on mitochondrial functions and morphology.

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Keywords: mitochondria, chemical chaperones, neurodegeneration

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DESIGN AND CONSTRUCTION OF EXPRESSION PLASMIDS FOR THE PRODUCTION OF CANNABIGEROLIC ACID IN YEASTS

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Abstract: Cannabinoids are bioactive proteins, which are known to the general public mainly due to their psychotropic effect. Withing the biotechnological and pharmaceutical sphere, cannabinoids represent a group of highly effective substances that have potential to treat symptoms of different diseases such as epilepsy. Research of these effects is however hampered down by legal definitions of cannabinoids in different states. Scientists are therefore driven to create an alternative pathway for production of cannabinoids in microbial systems. Expression in yeasts represents an excellent alternative for the *in planta* pathway. Therefore, in our work we aimed to create specific constructs which will allow us to produce cannabigerolic acid (CBGA) in yeasts *Candida utilis* and *Pichia pastoris*. We chose these yeasts based on their easy genetic manipulation, quick adaptation to different carbon sources, and fast growth on various medias. We successfully designed two genes named *CPT4-T* (gene for *C. utilis*) and *PPT4-T* (gene for *P. pastoris*). These genes which encode prenyltransferase are enriched with restriction sites for specific restriction endonucleases – *SalI*-HF and *SnaBI* for *C. utilis,* and *AgeI*-HF and *BstBI* for *P. pastoris*. Our genes of interest are placed on cloning plasmids named pUC57+*CPT4-T* (for *C. utilis*) and pUC57+*PPT4-T* (for *P. pastoris*). Initial electroporation of plasmid pUC57+*CPT4-T* into competent cells *Escherichia coli DH5α* has been successful, and we haven't encountered any issues. However, in case of plasmid pUC57+*PPT4-T*, we experienced issues with toxicity of our designed construct against host cells. Therefore, we had to repeat the electroporation process with multiple lines of competent cells such as *E. coli DH5α*, *XL1*, *C41*. At last, the electroporation process was successful when we chose competent cells from line *E. coli TG90*. Since these cells have the ability to transform high-copy plasmids into low-copy ones if ColE1 replicon is present, we chose to verify isolated clones with sequencing analysis using universal primers MF13. Sequencing analysis confirmed the authenticity of our sequences. After overcoming these issues, we isolated these cloning plasmids containing our genes of interest. We dissected them with previously mentioned restriction endonucleases. This step resulted in a 984 bp fragment that represents gene *CPT4-T* or *PPT4-T*. We ligated these fragments with specific expression vectors which resulted in a creation of two expression plasmids named pGC1 (for *C. utilis*) and pGP1 (for *P. pastoris*). These plasmids will be inserted into the chromosomes of our chosen yeasts. Verified clones will later on undergo an expression analysis by qPCR as well as functional analysis.

Keywords: cannabigerolic acid, cannabinoids, *Candida utilis*, *Pichia pastoris*, expression plasmids.

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THE EFFECT OF RADIOTHERAPY ON THE LEVEL OF INTRACELLULAR VITAMIN C IN PATIENTS WITH PROSTATE CANCER.

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Abstract: Prostate cancer ranks second among men in incidence and mortality due to malignant neoplasms. Radiotherapy together with oncological surgery are the primary method of treating prostate cancer patients.
The interaction of ionizing irradiation with water is responsible for producing reactive oxygen species (ROS). ROS interaction with cellular components may damage biomolecules, including DNA. This, in turn, may be responsible for cancer cell death. Taking into consideration the millimolar concentration of ascorbate in tissue, it is possible that in prostate cancer cells with reduced antioxidative protection, ascorbate (after supplementation) may act synergistically with ROS generated during radiotherapy and in this way to potentiate the therapy efficacy.
The study was performed on leukocytes obtained from men with prostate cancer undergoing radiotherapy and divided into two groups: supplemented by vitamin C and non-supplemented (control group). The intracellular vitamin C concentration level was determined by two-dimensional ultraperformance liquid chromatography with tandem mass spectrometry (2D-UPLC-MS/MS).
We observed a decrease in the level of intracellular vitamin C in patients after radiotherapy ($p<0.05$) without differences statistically significant between the supplemented and non-supplemented group.

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Keywords: RADIOTHERAPY, VITAMIN C, PROSTATE CANCER

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CHARACTERIZATION OF THE LATE STEP OF AURICIN BIOSYNTHESIS IN *STREPTOMYCES LAVENDULAE* SUBSP. *LAVENDULAE* CCM 3239

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Abstract: The soil bacteria streptomycetes are dominant producers of bioactive natural products with a wide spectrum of biological activities. A large number of these products belong to aromatic polyketides, which are synthesized by type II polyketide synthase (PKS). Although a large repertoire of aromatic polyketides has been identified, they all belong to only a few common structural types, including pyranonaphthoquinones, tetracyclines, angucyclines, anthracyclines, tetracenomycines, aureolic acids. We previously identified the *aur1* biosynthetic gene cluster (BGC) in *Streptomyces lavendulae* subsp. *lavendulae* CCM 3239, which was similar to anguclycline BGCs and was responsible for the production of antibiotic auricin. Interestingly, auricin is transiently produced in a narrow time interval after entering the stationary phase. This unusual pattern of auricin production is the result of its complex regulation by several regulators located in the BGC. Structural analysis of auricin revealed that it has interesting structural features that distinguish it from other known angucyclines. It is modified with D-forosamine and contains a unique spiroketal pyranonaphthoquinone-like aglycone similar to griseusin. In addition to being active against Gram-positive bacteria, auricin showed mild cytotoxicity against human tumor cell lines. The *aur1* BGC has an unusual organization. It consists of a central region containing homologous genes for angucycline. However, several putative auricin biosynthetic genes are scattered up to 30 kb from this region. One of them, *sa48*, encoding a polyketide cyclase/dehydratase homologue, is located on an operon under the control of the auricin-specific activators Aur1PR3 and Aur1PR4. To investigate its role, it was deleted in *S. lavendulae* CCM 3239. The resulting mutant did not produce auricin. Instead, a new yellow compound SA48A with a mass of 402.09763 was produced. However, this compound was unstable and it was gradually changed to another yellow compound SA48B with a mass of 400.08239. We optimized the isolation of SA48B and isolated the pure product, which we subjected to NMR structural analysis. The structure of SA48B corresponds to the auricin aglycone (with the loss of the sugar component D-forosamine). Based on these results, we hypothesize that the enzyme encoded by the *sa48* gene

encodes a cyclase necessary for the lactonization (and closure) of ring D. In its absence, a labile product SA48A with an open ring D is formed, which cannot by recognized by the glycosyltransferase to be modified by D-forosamine. However, after its export from the cell, it is gradually lactonized to SA48B. We characterized the biological properties of SA48B. It was much more active against Gram-positive bacteria and human tumor cell lines than auricin.

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Keywords: antibiotics, auricin, biosynthesis, *Streptomyces*

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PROFILING OF VOLATILE ORGANIC COMPOUNDS IN URINE TO MONITOR HUMAN HEALTH STATUS. A PILOT STUDY

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Abstract: Cancer is among the leading causes of mortality worldwide, emphasizing the urgent need for effective diagnostic strategies. The development of rapid, sensitive, and non-invasive screening techniques for early detection of carcinomas, which would increase the patient´s chances of survival, has thus become one of the significant challenges in 21st-century medicine. Among the promising methods is the analysis of volatile organic compounds (VOCs). VOCs are low-molecular-weight substances generated as final products of cellular metabolism. Their advantage lies in their emission from various biological matrices, including breath, urine, saliva, etc. Urine has the advantage of non-invasive collection, long-term storage, availability in large volumes, and VOC presence in relatively high concentrations. This work aims to optimize the protocol for profiling VOCs in urine samples using the BreathSpec instrument (G.A.S., Dortmund, Germany), which operates based on GC-IMS technology (Gas Chromatography-Ion Mobility Spectrometry). The strength of this spectroscopic method is the non-invasive real-time monitoring and identification of selected molecules. We focused on fundamental parameters that could influence the volatile profile: (i) the effect of temperature and time and (ii) the impact of the sample thawing method on the profile of released VOCs. We used urine samples from several relatively healthy donors for analyses. Consequently, we determined an optimized protocol with sufficient VOC quality for potential clinical application.

This study has been produced with the support of the Integrated Infrastructure Operational Program for the project: Research of spectroscopic methods for early, non-invasive real-time identification of selected diseases from the condensate of gases released by lungs and skin, ITMS: 313011BWX6, co-financed by the European Regional Development Fund.

Keywords: GC-IMS, urine, volatilome, metabolomics, spectrometry

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TRYPANOSOMATIDS WITH DISRUPTED RESPIRATORY CHAIN

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Abstract: Trypanosomatids a part of phylum Euglenozoa evolutionarily separated from other eukaryotes long time ago. Extended independent development is probably the reason why the metabolism of these protozoa contains many unusual metabolic pathways and structures. Among the most famous are the unique topology of mitochondrial DNA, glycolysis taking place separately from the cytoplasm in specialized organelles - glycosomes, RNA editing and the ability of aerobic metabolism even without the presence of cytochromes. While most features are common to all trypanosomatids described so far, the ability to live without heme-containing proteins is unique so far only to the trypanosomatid *Phytomonas serpens*. Another peculiarity of *P. serpens* is that it lacks complexes III and IV of the respiratory chain. However, in recent years, the loss of complexes III and IV has also been described in another trypanosomatid *Vickermania ingenoplastis*. A common feature of both mentioned species is that they lack the same genes for the subunits of the respiratory chain enzymes in both the nuclear and mitochondrial genomes.
In our project, we focused on the comparison of some biochemical parameters of *P. serpens* and *V. ingenoplastis*. We studied the activities of oxidative phosphorylation and Krebs cycle enzymes, oxygen consumption with different substrates as well as cross-reactivity with antibodies prepared against proteins from other trypanosomatids. Large differences in some of the observed parameters confirm results of phylogenetic analyses, which show that within the family of trypanosomatids, *P. serpens* and *V. ingenoplastis* are not closely related.
Supported by grants SK-CZ-RD-21-0038 and VEGA 1/0553/21.

Keywords: trypanosomatids, Phytomonas, Vickermania, bioenergetic metabolism

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INTERACTIONS OF THIOFLAVIN T WITH POLYSTYRENE SULPHONATE

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Abstract: It was shown that PSS promote the dye dimerization process. The fluorescent probe thioflavin T (ThT) is widely used for amyloid fibrils (AF) detection and testing. ThT exhibits properties of fluorescent molecular rotor and its emission intensity depends on microenvironment viscosity and rigidity. The binding of ThT to AF increases its fluorescence quantum yield by 2–3 orders of magnitude, which was explained by incorporation of ThT molecules into channels of the rigid β-sheet structure of the fibrils. Recently new and improved probes were designed for AF detection. Fluorescent probes with two emission bands are of particular interest for AF staining since they allow to develop a sensitive ratiometric method. Furthermore, in order to test amyloidosis in living cells and tissue, dyes that are sensitive to AF, such as ThT, but that absorb and fluoresce at longer wavelengths are required. The use of such a dye can decrease effects of both light scattering by AF in solutions (*in vitro*) and the absorption and fluorescence of biological tissues (*in vivo*). Fluorescence intensity enhancement in viscous solutions is related to decrease of the rate of twisted intramolecular charge transfer (TICT) process, associated with non-radiative deactivation of the excited state, due to restriction of the aromatic rings rotation relative each other. Incorporation of the probe molecule into β-sheet structures of AF results in restriction of the twisting movement of its fragments relative to each other, and this restriction significantly decreases the rate constant of transition to the non-fluorescent TICT-state resulting in enhancement of fluorescence intensity. However, the mechanisms by which ThT and its analogs are incorporated into amyloid fibrils and the stoichiometry of the dye–fibril complex are still actively debated. There are several points of view on the incorporation mechanism of ThT into amyloid fibrils. In particular, some researchers argue that ThT incorporates into fibrils in the dimer, excimer, or even micellar forms. Therefore, the solution of this problem is very important. It turned out that with the possible formation of fibrils from cytochrome c with PSS and monitoring their spectral changes with the addition of the fluorescent probe ThT, significant changes occur, which induces the formation of amyloid fibrils as we have already mentioned. We worked with different concentrations of cytochrome c and PSS and tried to confirm the results of our spectrofluorimeter measurements using DLS measurements of particle size on Zetasizers.

Keywords: thioflavin T, polystyrene sulphonate, amyloide fibrils

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Activation of a silent biosynthetic gene cluster for an unknown NRPS secondary metabolite in *Streptomyces lavendulae* subsp. *lavendulae* CCM 3239

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Abstract: *Streptomyces* is a genus of bacteria widely recognized for its key role in the production of medically important natural products, including antibiotics, antifungals, anticancer agents, and immunosuppressants. *Streptomyces* are known to produce more than two-thirds of the known antibiotics and are the primary source of antibiotics used in modern medicine. A large number of these products belong to cyclic peptides, which are mainly synthesized by non-ribosomal peptide synthetases (NRPSs). These biosynthetic enzymes are large modular multienzyme complexes responsible for the biosynthesis of diverse cyclic peptides found in bacteria, fungi, and plants. These complex enzymes have emerged as key players in drug discovery and biotechnology due to their ability to synthesize structurally complex and biologically active cyclic peptides that cannot be produced using traditional chemical methods. In general, biosynthetic genes for secondary metabolites in streptomycetes are physically grouped together with regulatory and resistance genes in so-called biosynthetic gene clusters (BGCs).
Genome sequencing and genomic analyses have revealed that the average *Streptomyces* strain contains approximately 30 BGCs, but only a small fraction of them is active under standard laboratory conditions, the rest of the BGCs are silent (or cryptic). Bioinformatic analyzes of the complete genome sequence of our model strain *S. lavendulae* subsp. *lavendulae* CCM 3239 revealed 30 silent BGCs that encode natural product pathways. Eight of them encode potential NRPSs. The aim of this work was to activate these BGCs, which were expected to encode NRPSs, by integrating the strong *kasOp** promoter upstream of the biosynthetic NRPS genes in the *S. lavendulae* CCM 3239 chromosome. To validate the strategy, we selected a silent BGC11 for putative NRPS, which contains four biosynthetic genes encoding dimodular NRPSs, located in an operon. DNA fragments surrounding the promoter region of the first gene in the operon, *SLAV_09235*, were amplified by PCR and cloned into a vector that was used to integrate the *kasOp** promoter into the chromosome by homologous recombination. Phenotypic analysis of the mutant *S. lavendulae*, *kasOp::9235* strain compared to WT revealed a dramatic overproduction of an antibacterial compound inhibiting the growth of *Bacillus subtilis*. These results confirmed the efficient activation of the silent BGC11 for NRPS using this system.

This work was supported by the Slovak Research and Development Agency under the contracts No. APVV-19-0009.
Keywords: antibiotics, cyclic peptides, NRPS, *Streptomyces*

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REPRESENTATION OF ssDNA IN GENOMIC DNA ISOLATES AND DNA-IP-Seq DATA

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Abstract: The inheritable genetic information is known to be well preserved within the stable DNA double helical structure, however, common processes as gene expression, DNA replication, and especially the repair of damages are accompanied by at least temporal and local destabilization and active metabolism. Upon such conditions, single-stranded DNA (ssDNA) structures might be available, protected by ssDNA binding proteins, but also exposed to DNA editing activities. We are interested in uracil-DNA metabolism that has importance in diverse biological processes as well as in anticancer therapy targeting thymidylate biosynthesis. In our research group, quantity and distribution of genomic uracil is being characterized in different models, where we faced with the limitations of the usual genomic DNA isolation and next generation sequencing (NGS) methods regarding the representation of ssDNA regions. Hence, we performed a comparative study about the ssDNA preserving capability of several commercially available genomic DNA isolation kits. We measured the ssDNA content of these isolates using dsDNase treatment and ssDNA specific Qubit assay. Furthermore, we also addressed the presence of RNA-DNA hybrids known to be accompanied by ssDNA structures. Then, we selected a method that was shown to well preserve the ssDNA component of genomic DNA isolated from HCT116 colon cancer cell line with inhibited uracil-DNA repair and treated by thymidylate synthase inhibitors. This is the same model on which we have previously characterized the drug induced genomic uracil patterns using our U-DNA-Seq method (PMID: 32956035). Since then, we have found that these two drug treatments resulted in similar but not the same genomic uracilation patterns. Several drug specific differences were also identified regarding the strictness of the replication arrest and the frequencies of cytosine deamination events. Now, using this well characterized model system, we performed a modified U-DNA-Seq on the ssDNA containing genomic DNA isolates. Our uracil-DNA sensor construct (PMID: 26429970) is able to bind uracilated DNA in single-stranded context as well, but we had to select a specific cleanup method to purify the precipitated DNA for sequencing. Similarly, we had to use a specific NGS library preparation method that allows integration of ssDNA fragments into the library. With the comparative analysis of these ssU-DNA-Seq data gained from both non-treated (low uracil content) and 5FdUR or RTX treated (high uracil content) samples, we can estimate the impact of ssDNA in this model which might be relevant for other functional genomic studies as well.

Keywords: U-DNA detection, ssDNA, RNA-DNA hybrids, DNA isolation, NGS

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SUPRAPHYSIOLOGICAL CONCENTRATIONS OF CHOLINE OXIDIZED IN MITOCHONDRIA SUPPORT THE FORWARD OPERATION OF ADENINE NUCLEOTIDE TRANSLOCASE WHEN COMPLEX I IS INHIBITED

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Abstract: The oxidation of choline to betaine aldehyde leads to the transfer of electrons to ubiquinone in mitochondria that express choline dehydrogenase (Cdh). This electron transfer supports complexes III and IV, thus generating the protonmotive force. Further catabolism of betaine aldehyde depends on CI activity due to NAD+ requirement. In complex I-inhibited, mouse liver mitochondria, the directionalities of adenine nucleotide translocase (ANT) and F1Fo-ATPase were estimated by the instantaneous effect of their respective inhibitors, carboxyatractyloside vs oligomycin, on membrane potential. In addition, matrix NADH levels were also recorded. Choline, at concentrations higher than those reported in physiological contexts in the literature, generated a sufficiently high mitochondrial membrane potential to sustain ANT operation in the forward mode in CI-inhibited mouse liver mitochondria. This was not observed when either CIII or CIV were inhibited. The directionality of the F1Fo-ATPase was unaffected by choline catabolism. The data show that Cdh-mediated choline catabolism could generate sufficient CIII and CIV proton pumping, thus supporting ANT operation in forward mode even under CI inhibition, but only if used at supraphysiological levels.

Keywords: choline dehydrogenase, adenine nucleotide translocase, reducing equivalent

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MITOCHONDRIAL DYSFUNCTION IN A HIGH INTRAOCULAR PRESSURE-INDUCED RETINAL ISCHEMIA MINIPIG MODEL

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Abstract
Introduction: Elevated intraocular pressure (IOP) is a major risk factor for developing glaucoma. It´s known that IOP can cause retinal ischemia (RI) with progressive neuronal death. It´s considered that altered mitochondrial dysfunction and processes such as fusion/fission and mitophagy may play role in pathophysiology of glaucoma.
Aim: This study focuses on changes in mitochondrial parameters due to elevated acute IOP in different porcine eye layers (RPE, neuroretina, choroid). In addition, the long term effects of the IOP were explored.
Material and Methods: The minipig eye RI model was developed by introducing of hyaluronic acid (HA) into the right eye´s anterior chamber, the left eye of each minipig served as a control. Cohort consists of 6 treated and 6 control eyes. Expression of selected genes (including genes responsible for michondrial dynamics) was determined by qPCR.
Results and discussion: *RPS18* showed to be most stable and was selected as reference gene. Results demonstrated decreased expression of *DRP1* in neuroretina and were consistent with our previous study (Pasak et al, 2022). Expression data will be correlated with mitochondrial functional and protein analyses.
Conclusion: The minipig eye mimics human and can be considered as suitable model to study glaucoma in context of mitochondrial parameters. Furthermore, there is evidence for the retinal impairment in various animal models also of other neurodegenerative diseases like Huntington‘s disease.

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References: Pasák M et al., Mitochondrial Dysfunction in a High Intraocular Pressure-Induced Retinal Ischemia Minipig Model. Biomolecules 2022
Keywords: retina, mitochondria, animal model, retinal ischemia, high intraocular pressure

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SANGER SEQUENCING SERVICE

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Abstract: Sanger sequencing is a fast and cost-efficient method for determining the primary sequence of short stretches of DNA. It has found a multitude of applications across scientific fields. Although this method has been largely replaced by NGS in recent years, it is still widely used for single gene sequencing in smaller-scale projects, for identifying genetic mutations associated with hereditary diseases, and in the verification of NGS findings.
We offer the possibility of using our Sanger sequencing service, provided under the auspices of the Comenius University Science Park in Bratislava, Slovakia. The process of Sanger sequencing involves a series of steps. Templates are amplified using chain termination PCR with fluorescently labeled ddNTP, and DNA fragments are subsequently separated on the ABI3500 genetic analyzer, generating sequencing data. In addition to sequencing analysis, we also provide fragment analysis of fluorescently labeled PCR products. All generated data undergo thorough inspection before being sent to the customer.
Our team comprises of experienced scientists in the field of sequencing who have participated in several domestic projects. For the last three years, we have performed an average of 5,400 sequencing and fragment analyses per year. We provide precise and reliable DNA sequencing solutions tailored to our customer′s research needs. We offer a personalized approach when optimizing the sequencing of samples and free consultations to ensure our services align with our customer′s project goals.

Keywords: Sanger sequencing, fragment analysis

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PROTEOMIC STUDYING OF EFFECTIVITY PHOTODYNAMIC INACTIVATION ON RICKETTSIAL INFECTIONS

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Abstract: Rickettsiae (Alphaproteobacteria; *Rickettsiales, Rickettsiaceae*) are small (0.3- to 0.5- by 0.8- to 2.0-μm) gram-negative obligate intracellular bacteria that target vascular endothelial cells and grow within the cytoplasm of eukaryotic host cells. Arthropods such as the ticks *Ixodes ricinus*, *Dermacentor reticulatus* and *D. marginatus* are vectors for rickettsioses causing spotted fever and the typhus groups of diseases in humans (Sekeyová, Z., Acta Virol, 2013).

The increasing prevalence of microbial infections and the rapid emergence of drug resistance to antibiotics created a critical health menace worldwide. Carbon quantum dots (CQDs) are a new type of fluorescent nanomaterial and are currently studied for use in photodynamic therapy (PDT) as an alternative for bacterial infections treatment. During PDT, CQDs generate superoxide, killing bacteria while mammalian cells remain intact. The cytotoxicity evaluation reveals that CQDs are bio- and blood-compatible in a wide therapeutic window (Rajendiran, K., Polymers, 2019).

In this work, we studied the protein differential expression occurred in *Rickettsia conorii* infected VERO cells subjected to PDT. Total proteins were purified from infected cells, trypsin digested and separated using an Ultimate LC System. The mass spectrometry was performed in a Waters Synapt system and for the differential expression analysis, the ProGenesis QI software was employed. Preliminary results showed an increasing of proteins involved in the *Rickettsia* lipopolysaccharide biosynthesis in the first 24 h after PDT and a downregulation of proteins involved in ATP syntesis and DNA repair at 48 and 72h after PDT. On the other hand, the VERO cells dysregulated proteins, included some related to detoxification and metabolism. Other observations included the VERO cells survival upon 72 h after infection and the lowering of *Rickettsia* replication rate in cells subjected to PDT.

To unveil the mechanisms by which intracellular bacteria contends with cellular damage from PDT and how the host cells can take advantage of a treatment to overcome an infection can help to develop improved anti microbial treatments to fight antibiotic resistance.

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Keywords: photodynamic inactivation, differential expression rickettsial infections.

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ACTIVE TARGETING OF CANCER CELLS BY MODIFIED TRANSPORT SYSTEM

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Abstract: Active tumor-targeting represents one of the great scientific challenges today. Compared to passive targeting, which is used to study anticancer drugs aimed at increased permeability and retention effect, active transport can specifically accumulate drugs at the tumor site and improve therapeutic efficacy. However, unlike passive transport, there is not only the possibility of increasing the therapeutic effect, but also minimizing the destructive effect on healthy cells and, ultimately, tissues and whole organisms. With the correct selection of the transporter, either a biocompatible nanoparticle of inorganic origin or a protein with an adequately bound active substance/drug, it is possible to effectively target tumor cells. Due to the properties of transporters, it is possible to use substances and drugs with different properties in targeted transport, whether hydrophobic or hydrophilic, photosensitive, cytostatic, etc. Here we present the results of the response of glioblastoma cells to active transport ensured by a transport system created on the basis of mesoporous and nanoporous structures of tetraethyl orthosilicate. Spherical biocompatible porous structures of silica were linked with a photoactive substance - hypericin - widely used in photodynamic therapy (PDT) against cancer. Hypericin is molecule characterized by its hydrophobic properties, thanks to which it is photoactive only in its monomeric form. In the water environment, its aggregates are formed and thus it becomes a photoinactive molecule. This feature was used to create our transport system. A hypericin molecule with an effective concentration was enclosed inside and on the surface of the particles. From the results of confocal fluorescence microscopy, it can be seen that in such a closed state the molecule is in an inactive state. However, by adding nanoparticles with hypericin to the cells, hypericin was diffused and monomerized in the cellular space, and at the same time, hypericin accumulated. This phenomenon was subsequently used for the application of photodynamic therapy. Light with a wavelength of 590 nm ensured the initiation of the internal pathway of apoptosis in cells with accumulated hypericin and, ultimately, the death of tumor cells due to the reactive oxygen species created. The efficiency of PDT using active transport was comparable to PDT with hypericin delivered directly to the cells. However, more in-depth studies are needed to increase the efficiency of cell targeting.

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Keywords: glioblastoma, apoptosis, ROS, silica nanoparticles

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APPROBATION OF PLATELETS AGGREGATION INHIBITOR FROM *ECHIS MULTISQUAMATIS* SNAKE VENOM ON ANIMAL MODEL *IN VIVO*

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Abstract
Background: The pursuit of new platelet aggregation inhibitors is an urgent issue, as today's World possesses new challenges: the emergence of patient resistance to antithrombotic agents, increased risk of blood loss, changing lifestyles, and genetic resistance to existing drugs. Platelets aggregation inhibitors from snake venoms are small proteins that bind to receptors on platelets surface with high affinity. Due to their specificity, these proteins don't interfering with coagulation factors, which means that platelets aggregation inhibitors from snake venoms have a lower risk of causing excessive bleeding. Also, because they are acting by targeting platelets receptors it helps to avoid the development of treatment resistance. Therefore, the aim of our study was to approbate platelets aggregation inhibitor from *Echis multisquamatis* snake venom (PAIEM) *in vivo*.
Methods: PAIEM was purified from crude venoms of *Echis multisquamatis* by ion-exchange and size-exclusion chromatography and analyzed by SDS-PAGE. PAIEM (0.22 mg/ml) was injected into the tail vein of female Wistar rats (n = 16) using a 0.3 ml syringe and a needle of diameter 30 g. Final concentration of inhibitor in blood plasma of animals was 10 μM. Rats were anesthetized by sodium thiopental. The procedures were conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine On the Protection of Animals from Cruelty No. 3447 of 21.02.2006. ADP- and collagen-induced platelet aggregation were used to study changes in the speed and degree of platelets aggregation.
Results & Discussion: After 1 hour of injection, ADP-induced aggregation of platelets was effectively inhibited. The speed and degree of platelet aggregation decreased on 80 and 47 % respectively compared to the control. The degree of collagen-induced platelets aggregation was decreased on 74 % compared to control. Final concentration of PAIEM in blood plasma was close to the IC50 determined in vitro. This can be an evidence of effective action of PAIEM and its resistance to natural inhibitors circulating in blood plasma. Most likely, as the most of platelet aggregation inhibitors from snake venoms PAIEM contains RGD-motifs that are recognized by integrin receptors on the surface of platelets. This allows inhibiting platelet aggregation process both quickly and effectively, independently on other factors.
Conclusions: Being injected intravenously PAIEM effectively inhibited platelet aggregation speed and degree. Thus, PAIEM can become an effective molecular basis for an antithrombotic agent that can more effectively prevent the formation of thrombosis.
Keywords: Disintegrin, snake venom, platelets aggregation inhibitor, animal model, thrombosis.

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THE GENOMIC SURVEILLANCE OF WASTEWATER CAPTURES THE DEVELOPMENT OF THE SARS-COV-2 VARIANTS IN SLOVAKIA'S POPULATION

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Abstract: Despite the declaration of the Coronavirus disease (COVID-19) pandemic three years ago, the World Health Organization still recognizes the pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a public health threat. While the global health emergency was lifted in May 2023, the systems established to manage the pandemic will remain in place. Early on, many states implemented surveillance systems to detect and monitor the SARS-CoV-2 virus. However, as the pandemic progressed and new variants emerged, clinical-based surveillance faced limitations such as testing capacity and costs. Wastewater-based (WWB) surveillance has emerged as a promising alternative to estimate the prevalence of circulating variants in the population. However, there are limitations due to the quality of isolated RNA and viral loads. To address these limitations, new bioinformatic tools and pipeline processing were developed to analyze WWB samples.

This study presents data collected from over 60 municipal wastewater collection facilities in the Slovak Republic from January to August 2022. We compared WWB data with clinical specimens to demonstrate how these two separate epidemiological approaches complement each other. Collecting sludge from all areas is a practical method to monitor the community's abundance and variant composition of the SARS-CoV-2 virus. WWB surveillance can be a crucial tool in the next epidemic or pandemic of human pathogens since the data generated by WWB are informative, similar to that gained by collecting and sequencing individual clinical samples.

Keywords: wastewater-based epidemiology, genome surveillance, infectious disease, public health

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A NEW SYNTHETIC BIOLOGY SYSTEM FOR INVESTIGATING THE BIOSYNTHESIS OF AROMATIC POLYKETIDE ANTIBIOTICS

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Abstract: Antibiotics belong to the most successful chemotherapy developed in the past. However, their extensive use inevitably leads to resistant microbes. Therefore, there is a constant and cyclical need for new efficient antibiotics against these multi-resistant bacteria. Antibiotics were discovered by screening natural products produced by bacteria and fungi for antibiotic activity. Over the past sixty years, a large number of different types of antibiotics have been discovered and characterized. However, this standard approach is currently rather limited to founding new efficient antibiotics. Current new technologies (molecular biology approaches, gene manipulations, genomics, and synthetic biology) open up exciting possibilities for the discovery of new drugs. In this regard, synthetic biology is a very promising approach. This emerging area can be defined as the design and construction of novel artificial biological pathways, organisms or devices, or the reengineering of existing natural biological systems for useful purposes. We used this strategy for the heterologous production of aromatic polyketides using the first landomycine biosynthetic genes from the landomycin biosynthetic gene cluster (BGC) of *Streptomyces cyanogenus* S136 responsible for the biosynthesis of landomycine aglycone. All nine genes (*lanA, lanB, lanC, lanF, lanD, lanL, lanE, lanM, lanV*) were amplified by PCR and inserted toto the expression plasmid pBSBR1 under the control of a strong *kasOp** promoter and an optimised RBS and terminated with a strong *fdT* terminator, producing monocistronic units *kasOp**-gene-*fdT*. Such gene cassettes were sequentially cloned and combined into the monocistronic artificial BGC *lanABCFDLE*, which was inserted into the PhiC31 phage-based pOri6 integration vector, resulting in pOri6-lanABCFDLE. After its conjugation into the heterologous host strain *S. coelicolor* M1146, we identified a high production of rabelomycin, which is a side intermediate of landomycin as well as most other angucycline antibiotics. Two additional cassettes containing *lanM* and *lanV* genes were inserted into the compatible PhiBT1 phage-based pOri12 integration vector, resulting in pOri12-lanM and pOri12-lanMV. After their conjugation into *S. coelicolor* M1146 with in pOri6-lanABCFDLE, we identified the production of dehydrorabelomycin (in the case of pOri12-lanM) and tetrangulol (in the case of pOri12-lanMV). These secondary metabolites are other intermediates in the biosynthesis of the landomycin aglycone. Hereby, we have validated and optimized this novel synthetic biology system based on monocistronic transcription units, which allows its use to combine genes from various aromatic polyketide pathways to produce new and modified secondary metabolites with potentially novel biological properties.

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This work was supported by the Slovak Research and Development Agency under the contracts No. APVV-19-0009.
Keywords: antibiotics, landomycin, synthetic biology, *Streptomyces*

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A SIMPLE AND HIGH-YIELDING APPROACH TO THE EXPRESSION AND PURIFICATION OF PSEUDOMONAS AERUGINOSA AZURIN

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Abstract: Azurin is a small periplasmic blue copper protein found in bacterial strains such as Pseudomonas and Alcaligenes where it facilitates denitrification metabolism. Azurin occurs naturally as a tetramer, with one metal cofactor in each monomer (128 amino acids, 14 kDa). Structurally, it has eight β-sheets arranged to a β-barrel structure and an α-helix that connects the fourth and fifth β-strands. Azurin is extensively studied for its ability to mediate the electron-transfer processes between enzymes associated with the cytochrome chain by undergoing oxidation-reduction between Cu(I) and Cu(II). Interestingly, azurin has also sparked the pharmaceutical community interest as a potential anticancer drug.
Here we present a novel protocol to express and purify azurin with high yields and appropriate metalation ratio. The fusion approach with an N-terminal GST tag was employed to obtain the pure protein without introducing further purification steps. After the on-column cleavage by PreScission Protease, the GPLGS-Azurin is collected and additionally incubated with copper sulphate to ensure sufficient metalation which was evaluated by measuring the A630/A280 ratio. The proper azurin folding was finally confirmed by circular dichroism spectroscopy.

Keywords: Azurin, blue copper protein, GST-tag, purification

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The pleiotropic anti-anti-sigma factor BldG is specifically dephosphorylated by the phosphatase SCO3691 to activate SigH and SigF pathway in *Streptomyces coelicolor* A3(2)

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Abstract: In their natural habitats, bacteria are exposed to various stresses. The stress response is regulated mainly by stress-response sigma factors of RNA polymerase, which govern the expression of genes necessary to overcome adverse conditions. In the Gram-positive *Bacillus subtilis*, the activity of such a sigma factor, SigB, is regulated by a partner-switching phosphorylation mechanism. Under non-stress conditions, SigB is sequestered by the anti-sigma factor RsbW. The release of the sigma factor from this complex is accomplished by the anti-anti-sigma factor RsbV, which is dephosphorylated by specific PP2C-type phosphatases RsbU/RsbP under stress conditions and sequesters RsbW. In addition, RsbW specifically phosphorylates RsbV through the serine protein kinase activity of its HATPase_c domain, providing negative feedback for SigB activation. Unlike *B. subtilis*, the Gram-positive soil bacterium *Streptomyces coelicolor* A3(2), which undergoes a complex cycle of morphological differentiation, contains nine SigB homologues (SigBFGHIKLMN) with a major role in differentiation and osmotic stress response. In addition, it contains 45 RsbW homologues, 17 RsbV homologues, and 44 RsbU/RsbP homologues, suggesting a rather complex regulation of these SigB homologues compared to *B. subtilis*. We previously characterized the sigma factor SigH, which has a dual role in regulating the osmotic stress response and morphological differentiation. Its activity is negatively regulated by its specific anti-sigma factor UshX, and the anti-anti-sigma factor BldG is involved in the osmotic stress activation of SigH by sequestering UshX. However, UshX lacks the HATPase_c domain and is unable to phosphorylate BldG. This negative feedback phosphorylation is carried out by the SigF-specific anti-sigma factor RsfA. Regulation is more complex because 13 other RsbW homologous anti-sigma factors interact with BldG and seven of them specifically phosphorylate BldG. One of them, SCO7328, was shown to interact with three sigma factors, SigG, SigK and SigM. These data indicate that BldG activates several SigB homologues in *S. coelicolor* A3(2). A critical signal for the activation of SigB homologues is transduced by the activation of a specific PP2C phosphatase. However, *S. coelicolor* A3(2) contains at least 44 RsbU/RsbP homologues. This suggests a complex interplay of different signals to activate specific anti-anti-sigma factors. None of these RsbU/RsbP homologues have yet been studied in *S. coelicolor* A3(2). We therefore developed an *in vitro* system to identify PP2C phosphatases that can dephosphorylate BldG-P. The system was tested with five selected PP2C phosphatases from *S. coelicolor* A3(2), and one of them, SCO3691, was found to specifically dephosphorylate BldG-P.

This work was supported by the VEGA grant 2/0026/20 from Slovak Academy of Sciences.
Keywords: anti-sigma factor, differentiation, sigma factor, *Streptomyces*

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THE INTRIGUING EFFECT OF CHOLESTEROL-BASED SURFACTANT CHOBIMALT ON INSULIN AMYLOID AGGREGATION: BIOPHYSICAL AND BIOCHEMICAL STUDIES

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Abstract: Self-assembly is based on autonomous, non-covalent interactions between distinct building blocks without requirements of external stimuli/energy sources. Although the proteins show sequence, size, and function diversity, they all form similar amyloid fibrils consisting of the same cross-β structure, i.e. β-strands arranged perpendicular to the long fibril axis. However, proteins differ significantly in their propensity and the conditions in which they form fibrils. Hydrophobic interaction of amyloid-prone proteins/peptides with membranes and/or with the individual membrane components is particularly important, since it is believed that membrane composition is one the factors controlling the aggregation process.[1,2] The importance of membrane surface/composition is also observed for insulin aggregation, especially when composition differs from the native pancreatic β-cell's membrane, where insulin is secreted. It appears that a significant role is played by cholesterol, which is typically absent in pancreatic β-cells. On the other hand, among amyloid-prone proteins, insulin attracts attention because of its physiological and therapeutic importance. The amyloid aggregation of insulin is studied in the presence of cholesterol-based detergent, Chobimalt. The strategy to elucidate the Chobimalt-induced effect on insulin fibrillogenesis is based on performing the concentration- and time-dependent analysis using a combination of different experimental techniques, such as ThT fluorescence assay, CD, AFM, SANS, and SAXS. The obtained results demonstrated a dose-dependent effect of cholesterol-based detergent, Chobimalt on the kinetic of insulin fibrillization and morphology of formed fibrils.[3] Depending on the protein to Chobimalt molar ratio we observed the dramatically changed kinetics and morphology of fibrillar aggregates. The fibrils appear to be more flexible and wavy-like with a tendency to form circles. [3] Amyloid aggregation requires the formation of unfolded intermediates, which subsequently generate amyloidogenic nuclei. We hypothesize that the different morphology of the formed insulin fibrils is the result of the gradual binding of Chobimalt to different binding sites on unfolded insulin. A similar explanation and the existence of such binding sites with different binding energies were shown

previously for the nonionic detergent. Thus, the data also emphasize the importance of a protein partially-unfolded state which undergoes the process of fibrils formation; i.e., certain experimental conditions or the presence of additives may dramatically change not only kinetics but also the morphology of fibrillar aggregates.

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Keywords: Chobimalt, cholesterol-based detergent, insulin, amyloid aggregation, fibrillar morphology

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PROTEOME AND PHOSPHOPROTEOME CHANGES DURING MITOTIC AND MEIOTIC CELL DIVISIONS IN *S. POMBE*

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Abstract: The combination of affinity enrichment strategies coupled with mass spectrometry represents an effective tool for analysis of post-translational modifications [1]. The aim of this study was to analyze the changes in the proteome and phosphoproteome of the fission yeast *S. pombe* during mitosis and meiosis I/II. For this purpose, we employed strains bearing a temperature-sensitive (pat1-ts/pat1-114) and a conditional ATP analog-sensitive (pat1-as2) alleles of the Pat1 protein kinase. Inhibition of the Pat1 by elevated temperature, or by ATP analog allows to induce highly synchronous meiosis in *S. pombe* [2]. In our phosphoproteomic strategy we combined TMTpro 18plex labeling, TiO2 and FeNTA enrichment of phosphopeptides, high pH RP fractionation, and LC-MS analysis. Using TMTpro 18 plex labeling in combination with phosphopeptide enrichment and fractionation followed by LC-MS analysis, we were able to quantify 4672 proteins and 7172 phosphosites of two mutant strains of the S.pombe. Among these, the expression level of 2680 proteins and the rate of phosphorylation of 4005 phosphosites changed significantly during the progression of cells through meiosis. These results establish a basis for further elucidation of the importance of particular proteins and their phosphorylation for the regulation of meiotic cell divisions in this yeast. In addition, it may explain the observed changes in meiotic division in the studied mutant strains, pat1-114 and pat1-as2.

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Keywords: proteomics, phosphoproteomics, mitosis, meiosis, *S.pombe*

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COMBINATION OF EXPERT GUIDELINES-BASED AND MACHINE LEARNING-BASED APPROACHES LEADS TO SUPERIOR ACCURACY OF AUTOMATED PREDICTION OF CLINICAL EFFECT OF COPY NUMBER VARIATIONS

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Abstract: Clinical interpretation of copy number variants (CNVs) is a complex process that requires skilled clinical professionals. General recommendations have been recently released to guide the CNV interpretation based on predefined criteria to uniform the decision process. Several semiautomatic computational methods have been proposed to recommend appropriate choices, relieving clinicians of tedious searching in vast genomic databases. We have developed and evaluated such a tool called MarCNV and tested it on CNV records collected from the ClinVar database. Alternatively, the emerging machine learning-based tools, such as the recently published ISV (Interpretation of Structural Variants), showed promising ways of even fully automated predictions using broader characterization of affected genomic elements. Such tools utilize features additional to ACMG criteria, thus providing supporting evidence and the potential to improve CNV classification. Since both approaches contribute to evaluation of CNVs clinical impact, we propose a combined solution in the form of a decision support tool based on automated ACMG guidelines (MarCNV) supplemented by a machine learning-based pathogenicity prediction (ISV) for the classification of CNVs. We provide evidence that such a combined approach is able to reduce the number of uncertain classifications and reveal potentially incorrect classifications using automated guidelines. CNV interpretation using MarCNV, ISV, and combined approach is available for non-commercial use at https://predict.genovisio.com/.

Keywords: copy number variation, machine-learning

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DNA INTERACTIONS OF NEW METAL COMPLEXES WITH 2-THIOPHENE CARBOXYLATE LIGANDS

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Abstract: Recently, there has been focus on the development of small molecules designed to target a deoxyribonucleic acid for therapeutic purposes. These low weight molecules generally interact with DNA through various covalent and non-covalent interactions (intercalation, groove binding, external binding).
Several metal-based anticancer drugs are currently being tested in clinical trials. Such metal complexes have ability to effectively bind or cleave DNA. Many silver compounds can interact with DNA and have various biological effects such as anticancer, antibacterial, antifungal, etc. Also zinc complexes exhibit antimicrobial and anticancer activity and they have shown the DNA binding properties. Gallium compounds have antimicrobial properties and, as well as zinc and silver compounds, are able to interact with DNA.
The aim of this work was to investigate DNA binding potency of new metal complexes with 2-thiophene carboxylate ligands (2-Tio-COO): $[Ag(2\text{-Tio-COO})]_n$ (**1**), $[Zn(2\text{-Tio-COO})_2]_n$ (**2**) and $[Ga(2\text{-Tio-COO})_3]\cdot H_2O$ (**3**). Non-covalent interactions of the newly synthesized complexes with DNA were investigated using the methods such as UV-vis, fluorescence and CD spectroscopy. From spectral measurements, we have established the Stern–Volmer binding constants (K_{sv}). K_{sv} for displacement of ethidium bromide from DNA–EB complex were in the range 0.648 – 3.420×10^3 M^{-1} and for displacement assay with Hoechst 33258 were in the range from 0.3095×10^3 to 3.823×10^3 M^{-1}.

Keywords: DNA, non-covalent interactions, metal complexes

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EFFECT OF CRYPTOPLEURINE ON LEUKEMIC CELL LINES WITH DIFFERENT P-GLYCOPROTEIN EXPRESSION

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Abstract: Leukemia is a type of cancer that affects the blood and bone marrow and causes excessive production of abberantly differenciated leukocytes. A major challenge in the treatment of not only leukemia, but all types of malignancies is to reverse multidrug resistance. This gives malignant cells the ability to resist chemotherapeutic agents with different structures and mechanisms of action. ABC transporter-mediated multidrug resistance is one of the most important causes of treatment failure in hematologic malignancies. The most studied ABC transporter is P-glycoprotein, whose increased expression is one of the major markers of multidrug resistance and is frequently found in acute leukemia. For these reasons, new therapeutics are constantly being sought, and alkaloids of natural origin, which are being intensively studied for their therapeutic properties, have shown very promising potential. Therefore, in this work, we focused on acute lymphoblastic and myeloid leukemia cell lines that also express P-glycoprotein. We investigated the effect of the plant alkaloid cryptopleurine, which belongs to a group of phenanthroquinolizidines characterized by diverse biological activities. Their properties include, for example, anti-inflammatory, antiviral and antimicrobial effects. We used the colorimetric MTT assay to determine the cytotoxic effect of cryptopleurine. The next step was to determine the type of cell death using flow cytometry and electrophoretic analysis of DNA fragmentation. Using the JC-1 fluorescent probe, we assessed changes in mitochondrial membrane potential, a marker of cell death. Since cruptopleurin is thought to influence the cell cycle, we observed modifications in its course.

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Keywords: Leukemia, P-glycoprotein, multidrug resistance, cryptopleurine

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STRUCTURAL AND FUNCTIONAL EFFECTS OF DISEASE-RELATED POINT MUTATIONS IN THE RNA BINDING REGION OF KMT2D

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Abstract: KMT2D (MLL4) is a histone methyltransferase responsible for the monomethylation of the H3K4 residue in active enhancer regions which is essential for the proper regulation of cell-type specific gene expression and differentiation. Mutations of the KMT2D gene have been implicated in the development of Kabuki-syndrome, a serious genetic disorder leading to several developmental defects. While Kabuki-related KMT2D mutations generally lead to the loss of a significant portion of the protein, resulting in an enzymatically inactive variant, other, seemingly less-disruptive missense mutations have also been implicated in the emergence of serious developmental defects. These missense variations exclusively localize to a short segment of KMT2D, a region with RNA binding capacity and a predicted coiled-coil formation tendency, and do not affect the enzymatically active SET domain on the C-terminus. In order to understand how these mutations interfere with the normal activity of KMT2D, we expressed the affected region (RBR-Q) and compared the behaviour of eleven disease-related variants with the wild type. Microscale thermophoresis, temperature dependent translational diffusion NMR and circular dichroism spectroscopy results indicated that the wild type RBR-Q region has a predominantly alpha helical structure and possesses a significant tendency to self-associate. The mutations interfere with the native structure of RBR-Q and reduce the self-association capacity, but not in a uniform manner. They also appear to influence the RNA binding capacity of the protein, as the mutants had altered affinity and specificity towards the tested RNAs.

Keywords: RNA binding, coiled-coil, NMR, circular dichroism, oligomerization

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OVEREXPRESSION OF GRP78/BIP IS RESPONSIBLE FOR ALTERED RESPONSE OF CELLS TO TUNICAMYCIN AS A STRESSOR OF THE ENDOPLASMIC RETICULUM IN P-GLYCOPROTEIN-POSITIVE L1210 CELLS

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Abstract: P-glycoprotein (P-gp), member of the ABC (ATP-binding cassette) transporter family localized in leukemia cell plasma membranes is known to reduce cell sensitivity to a large but well-defined group of chemicals known as P-gp substrates. However, we found previously that P-gp-positive sublines of L1210 murine leukemia cells (R and T) but not parental P-gp-negative parental cells (S) are resistant to the endoplasmic reticulum (ER) stressor tunicamycin (an N-glycosylation inhibitor). Here, we elucidated the mechanism of tunicamycin resistance in P-gp-positive cells. We found that tunicamycin at a sublethal concentration of 0.1 μM induced retention of the cells in the G1 phase of the cell cycle only in the P-gp negative variant of L1210 cells. P-gp-positive L1210 cell variants had higher expression of the ER stress chaperone GRP78/BiP compared to that of P-gp-negative cells, in which tunicamycin induced larger upregulation of CHOP (C/EBP homologous protein). Transfection of the sensitive P-gp-negative cells with plasmids containing GRP78/BiP antagonized tunicamycin-induced CHOP expression and reduced tunicamycin-induced arrest of cells in the G1 phase of the cell cycle. Taken together, these data suggest that the resistance of P-gp-positive cells to tunicamycin is due to increased levels of GRP78/BiP, which is overexpressed in both resistant variants of L1210 cells.

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Keywords: multidrug resistance; p-glycoprotein; tunicamycin-induced ER stress; GRP78/BiP; CHOP

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HETEROCYCLIC COMPOUNDS OF SYNTHETIC ORIGIN AS A TOOL IN THE TREATMENT OF CANCER

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Abstract: Haematological malignancies represent a significant proportion of mortality in developed countries. One of the major obstacles to the treatment of haematological malignancies is the mechanism of multiple drug resistance mediated by ABC transporters. ABC transporters function as efflux pumps that block the loading of cells with cytotoxic agents. One of the most abundant ABC transporters that has been most extensively studied to date is p-glycoprotein (P-gp), also known as the multidrug resistance protein. Numerous research teams are trying to find a potent inhibitor of P-gp or a potent cytotoxic drug that is not a substrate of P-gp, either naturally occurring or synthetically produced.
The aim of this work was to investigate the effects of five newly synthesized 5-aminopyrazole derivatives (1) on acute myeloid leukemia or acute lymphoblastic leukemia cell lines. In the first part, we focused on the cytotoxic effect of the derivatives evaluated by MTT assay and detected the rate of apoptosis or necrosis induced by these compounds using flow cytometry . In addition, we detected the formation of autophagolysosomes using monodansyl cadaverine by confocal microscopy. We analysed changes in mitochondrial membrane potential, an indicator of cell death, using the fluorescent probe JC-1 .

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Keywords: cancer, aminopyrazole, P-glycoprotein, MDR

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FUNDAMENTAL ROLE OF MITOCHONDRIAL CARBOXYLATION IN SUPPORTING THE METABOLISM OF HUMAN BRAIN CELLS

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Abstract: All three biotin-containing mitochondrial carboxylases, namely: pyruvate carboxylase (PC), propionyl-CoA carboxylase (PCC), and 3-methylcrotonyl-CoA carboxylase (MCC); are expressed among the cells in human brain parenchyma. The both, PC and PCC, are suggested to possess the anaplerotic role. PC can utilize pyruvate to convert it to oxaloacetate, while substrate for PCC is propionyl-CoA. MCC catalyzes the conversion of 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA, the intermediates in the irreversible part of leucine catabolism. Predominate source for pyruvate could be breakdown of the glucose, while propionyl-CoA is an intermediate common for catabolism of Val, Ile, Met, Thr, and odd-chain fatty acids. End products of PC or PCC catalyzed reaction is oxaloacetate or succinyl-CoA, respectively, the intermediates of Krebs cycle. Since MCC shifts carbon-skeleton originating from Leu to generation of acetoacetate and acetyl-CoA, together with PCC could contribute to catabolism of five essential amino acids. In this respect, the anaplerotic function of PC and PCC contributes to de novo synthesis of Glu and Asp and other non-essential amino acids. In addition, those enzymes are involved in sustaining of several neurochemical processes to support neuronal physiological functions, such as Glu/Gln cycle. With the aim i) to study the cell-type specific expression of mitochondrial carboxylases in brain cells either in culture or in situ, ii) to evaluate their role in supporting metabolism of cultured astrocytes and brain tumor cells and iii) to identify amino acids those can serve the role of metabolic substrates; set of metabolomic, immunoprobing, biochemical and histochemical experiments was performed. Our results show that all three mitochondrial carboxylases are expressed in human brain and cultured astrocytes, astrocytoma, glioblastoma and neuroblastoma cells. Metabolomic analysis of culture media revealed that cells readily removed glucose, glutamine and branched-chain amino acids, while cancer cells enriched media with several compounds including citrate and phenylalanine. Release of citrate from cancer cells could be underlaid by sufficiently active processes of anaplerosis and acetyl-CoA generation. Indeed, we revealed that inhibition of PC is lethal for cells. Application of ^{13}C-Leu revealed the isotopic enrichment of citrate mass to M+2, which stress the capacity of cells to catabolize leucine. This could be confirmed by detection of MCC expression, which is an enzyme specific for irreversible part of Leu catabolism. Additionally, disappearance of Ile and Val from media point to possibility of PCC presence that is essential for their catabolism. The rise of Phe level in media from growing cancer cells evokes that in addition to free amino acids they consume either peptides or proteins from their milieu. Indeed, FITC labeled albumin was used to monitor the capability of cancer cells to engulf the extracellular proteins and to monitor its intracellular fate. Such results indicate that brain cancer cells might employ on uptake of extracellular proteins as an additional

source of amino acids for their metabolism. In scope of translation research, we confirmed the expression of MCC and PC among human astrocytoma, glioblastoma and oligodendroglioma samples by immunoprobing of obtained dissected tumors. Furthermore, we revealed PC is expressed not only in astrocytes but also in neurons in human brain.

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Keywords: metabolism, anaplerotism, pyruvate carboxylase, propionyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase

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FINGOLIMOD AS THE DUAL MODULATOR OF DEATH AND DIFFERENTIATION OF ACUTE MYELOID LEUKAEMIA CELLS

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Abstract: Sphingolipids represent one the major lipid species found in eukaryotic cells and their role as bioactive molecules has been elucidated over the last several years. Namely, sphingolipids have been proven to regulate various cellular processes such as cell adhesion, migration, proliferation, senescence, death, or differentiation. Indeed, the dysregulation of sphingolipid metabolism or sphingolipid-mediated signalling is often found in cancer cells, supporting the uncontrolled tumour growth, drug resistance of cancer cells, suppression of immune responses, or angiogenesis. Therefore, novel drugs targeting sphingolipids, their metabolism or signalling pathways are researched as promising anticancer therapeutics.

Fingolimod (also known as FTY720) is a synthetic analogue of sphingosine and is employed as an immunosuppressive drug to treat multiple sclerosis. However, fingolimod has been also studied as a potential antineoplastic drug owing to its ability to modulate cancer cell metabolism and signalling via its multiple intracellular targets or four of five sphingosine-1-phosphate G-protein coupled receptors to promote inhibition of the cell cycle progression, induction of apoptosis or inhibition of tumour-associated angiogenesis.

In our work, acute myeloid leukaemia (AML) cells were employed, namely cell lines SKM-1 and MOLM-13 and their sublines established by vincristine selection, referred to as SKM-1/VCR and MOLM-13/VCR, displaying multidrug resistant (MDR) phenotype due to P-glycoprotein overexpression. We found out that LC50 concentrations for SKM-1 and SKM-1/VCR cells are comparable (ca. 6 μM) while MOLM-13/VCR cells are slightly more sensitive to fingolimod than MOLM-13 cells (LC50 ca. 6 μM vs. 8 μM). These results indicate that fingolimod can circumvent MDR of AML cells. Moreover, fingolimod acts as a potent inducer of apoptosis, demonstrated by dual annexin V-FITC and propidium iodide staining, DNA fragmentation and caspase-3, 7, 8 and 9 activities, with the maximum effect observed after 8 hours. This is also accompanied by the decrease in mitochondrial membrane potential (MMP). Noteworthy, even sublethal concentrations of fingolimod can lead to the decrease in MMP, generation of ROS and increase in intracellular Ca^{2+} concentration, the effects similar to 30 nM phorbol-12-myristate-13-acetate, a known inducer of AML cells differentiation. Taking this into account, the upregulation of differentiation markers CD14 and CD11b assessed by qPCR indicates that fingolimod applied in sublethal concentrations for two weeks promotes the differentiation of all tested cell lines, opening new possibilities for the use of this drug in differentiation-based anticancer therapy which requires further research.

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Keywords: acute myeloid leukaemia, fingolimod, apoptosis, cell differentiation

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THE IMPORTANCE OF SIALIC ACID FOR TICKS AND TICK CELL LINES

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Abstract: Sialic acids (Sias) belong to a family of sugars that play important roles in various biological processes. Sias are commonly found on the surfaces of cell membranes, and as a typical glycan of vertebrates, they can participate in cellular communication, immune response, neurogenesis and pathogen recognition/transmission. Moreover, many pathogens and parasites display Sia on their surface as a protection from the immune system of their hosts. The presence and role of Sia in ticks and other invertebrates are not well described. It has been identified in several insect species with a proven role during early-stage development (Drosophila melanogaster), and its possible role in dengue virus-vector interaction has been proposed (Aedes aegypti). To shed light on this issue in ticks, we used N-azidoacetyl-D-mannosamine as a sialic acid precursor and Click chemistry reaction to mark and detect newly synthesized sialylated glycans in ticks and tick cell lines. Using in vitro feeding, we traced sialic acid from fed females through eggs to larvae.

Keywords: xialic acid, tick, Ixodes ricinus, tick cell line, click chemistry

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BASIC CHARACTERIZATION OF STAPHYLOKINASE VARIANTS EVOLVED BY RIBOSOME DISPLAY

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Abstract: Thrombosis, which refers to the formation of blood clots in specific areas, has a considerable medical impact. The acute arterial thrombosis is the main underlying factor for the majority of myocardial infarction (heart attacks) and approximately 80% of strokes. Collectively, these conditions represent the leading cause of mortality in developed countries. (Mackman, 2008). Prompt treatment with thrombolytic drugs can restore blood flow before major brain damage has occurred and improve recovery after stroke (Wardlaw et al., 2014). An ideal thrombolytic agent is expected to prevent re-occlusions, has higher fibrin specificity, decrease bleeding complications, be retained in the blood for a longer time to minimize dosage and be less antigenic for repetitive usage. However, thrombolytic drugs have shown limited efficacy and notable hemorrhagic complication rates, offering potential for enhancement (Nikitin et al., 2021). Bacterial staphylokinase (SAK) is a small-size plasminogen activator, which hinders the systemic degradation of fibrinogen and reduces the risk of severe hemorrhage. It has a high tendency to make fibrin-bound plasminogen turn into plasmin through formation of equimolar (1:1) complex with plasmin, which can activate inactive zymogen plasminogen to its active form, plasmin. SAK is considered to be a promising thrombolytic agent with properties of cost-effective production and negligible side effects (Nedaeinia et al., 2020).
In order to enhance SAK´s effectiveness and to minimize potential adverse effects, it is crucial to enhance its affinity and selectivity towards plasmin. This can be achieved through the method of directed evolution of proteins, a highly effective method for customizing protein properties to achieve new or improved functionalities. In our study, we utilized ribosome display, to evolve new SAK variants, thus modifying SAK protein characteristics. Here we provide basic characterization of the variants obtained through the aforementioned method. In addition, we outline the fundamental biophysical properties and explain the purification process of certain mutant forms.

Keywords: thrombosis, thrombolytics, staphylokinase, ribosome display

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LACK OF ANTIBODY VALIDATION CHALLENGES CANCER RESEARCH TRANSLATION AND REPRODUCIBILITY: A CASE OF CA IX

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Abstract: Translation of cancer research to clinical success has been remarkably low and clinical trials in oncology have the highest failure rate compared to other therapeutic areas (Begley and Ellis, doi: 10.1038/483531a). This high failure rate is related to many factors including inadequate pre-clinical tools and high proportion of published data with low reproducibility rate (see the reference above). Therefore, it is crucial to pay attention to controls, reagents, investigator bias and description of the complete data set. The demand for the scientific rigour and integrity is in cancer research particularly important, because of a very high complexity of this disease, not only due to many different sites of origin, types and subtypes, interindividual differences in pathologies and therapy responses, but also due to intratumoral heterogeneity shaped among other factors by tumor tissue microenvironment. During cancer progression, cancer cells are exposed to multiple physiological stresses present in the growing tumor tissue, which generate selection pressures and adaptive responses leading to expansion of cell subpopulations able to survive and sustain proliferation. These subpopulations exhibit phenotypic and metabolic plasticity and invasive/pro-metastatic behaviour, and contribute to heterogeneous tissue architecture with various physiological gradients. Understanding mechanisms behind these phenomena requires not only technologically advanced high-throughput metabolomic, proteomic, and genomic approaches, but also classical methods of molecular and cellular biology. The latter approaches have already uncovered spectrum of molecules and pathways that often display highly heterogeneous expression patterns reflecting dynamically changing selection-adaptation forces in tumor microenvironment. Using the example of carbonic anhydrase IX (CA IX), which is a cancer biomarker under development for therapeutic targeting, we provide a basic insight into the current challenges of investigation of tumor heterogeneity in experimental cell models and in tissue specimens. Since CA IX expression pattern reflects gradients of oxygen, pH, and other intratumoral factors, it can be used as a paradigm to discuss an impact of antibody and research material quality and methodological rigour on resulting data, their interpretation and reproducibility. Based on the validation, we propose the most reliable CA IX-specific antibodies and suggest general conditions for faithful immunohistochemical analysis of molecules that are implicated in cancer biology and display potential for translation to clinic.

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Keywords: validation, tumor microenvironment, tumor heterogeneity, carbonic anhydrase IX

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DIRECTED EVOLUTION OF A STAPHYLOKINASE, A THROMBOLYTIC DRUG, BY RIBOSOME DISPLAY

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Abstract: Directed evolution represents a powerful strategy for the development of therapeutic proteins with improved properties [1]. We employed ribosome display to improve a thrombolytic agent, staphylokinase (SAK). The usage of currently available thrombolytics is limited due to their high immunogenicity and hemorrhagic complications. SAK is a single-chain extracellular protein secreted by *Staphylococcus aureus*. It initiates the fibrinolytic cascade to help invading bacteria move deeper into the tissues. Owing to its thrombolytic properties and high fibrin specificity, it is considered a promising new thrombolytic agent. However, not all SAK features are optimized for their practical applications, leaving room for improvement. Engineering the affinity and stability of SAK could increase its residence time on plasmin, thus reducing the severity of side effects.

We have successfully implemented the selection of SAK variants towards plasmin by ribosome display and after the first round of display we proved that it is feasible to evolve SAK by this technology. Evolution techniques usually require several consecutive rounds; therefore, we accomplished six rounds of the display. Sequencing of individual clones after consecutive rounds of ribosome display revealed hot spot amino acid substitutions, indicating that such residues might be involved in SAK-plasmin interaction and its replacement might change SAK property towards higher plasmin affinity.

We expressed and purified several selected SAK mutants after the 5th and 6th round of ribosome display and tested their activity. Preliminary data obtained from SAK engineered by ribosome display showed that the evolution approach has a high potential to improve the properties of SAK. However, we need to perform detailed biophysical characterisation of selected mutants to prove this statement.

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Keywords: directed evolution, ribosome display, staphylokinase, thrombolytic drug

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POTENTIAL USE OF INSULIN-LIKE GROWTH FACTOR 2 SELECTIVE ANALOGUES IN TREATMENT OF NEURODEGENERATIVE DISEASES

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Abstract: Insulin, insulin-like growth factors 1 and 2 (IGF1 and IGF2) and their respective receptors (IR-A, IR-B, IGF1R and IGF2R) play a key role in human physiology and disease. They form a complex network regulating homeostasis, development, and growth of the organism. IGF2, which is in the adult organism several times more abundant than both IGF1 and insulin, can bind to the receptors for insulin, IGF1, and additionally to its receptor, IGF2R. It is known to have a carcinogenic effect when acting through IR-A or IGF1R. On the other hand, clearance of IGF2 from the circulation via IGF2R also has an anti-cancer role. The effect of IGF2 on memory consolidation when signaling through IGF2R has also been described. IGF2 deficiency in the brain is associated with the progression of neurodegenerative diseases. Therefore, IGF2 could have therapeutic potential.
While treatment of certain cancers requires a reduction in IGF2 levels, treatment of neurodegenerative diseases benefits from an increase in IGF2 levels. The aim of this work was to design, prepare and characterize selective IGF2 analogues with increased affinity for IGF2R and reduced affinity for IGF1R and IR-A so that they would exert neuroprotective effects and have significantly suppressed carcinogenic effects at the same time.
The IGF2 analogues were designed to have mutations in the sites responsible for binding to IGF2R. They were produced using the E. coli expression system. Purified proteins were tested in an *in vitro* affinity binding assay for affinity for IGF2R, IGF2R domain 11 (D11), IGF1R, IR-A and IR-B. Out of our prepared analogues, there are several that have reduced binding to IGF1R yet retained or increased binding to IGF2R. These analogues, for which we plan to move to *in vivo* testing, could be useful for improving memory in some neurodegenerative diseases.

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Keywords: IGF2, IGF2 analogs

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LIQUID-LIQUID PHASE SEPARATION OF A BACTERIAL TRANSLATION FACTOR

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Abstract: Compartmentalization is a hallmark of living cells that allows them to perform complex tasks by dynamically coordinating matter and energy fluxes in space and time[1]. This compartmentalization of membrane-less organelles in prokaryotes is driven by Liquid-Liquid Phase Separation (LLPS)[2]. Studies have shown LLPS to be a major driving force in the subcellular organization of bacterial cells[3]. These "biomolecular condensates" are comprised of proteins that are generally rich in intrinsically disordered regions (IDRs)[4].
In prokaryotes, translation initiation factor 2 (IF-2) is a GTPase that binds the initiator tRNA and catalyses the ribosomal subunit joining to form the elongation competent 70S complex[5]. A large portion of IF-2 contains IDRs, making the protein a favourable candidate for homotypic and/or heterotypic interactions.
Here, we present biochemical evidence that IF-2 can phase separate under specific conditions. The IF-2 LLPS formation can provide deeper insight into compartmentalized translation machinery in bacterial cells.

Keywords: Phase separation, translation, Initiation Factor 2, condensate

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CLONAL COMPETITION AND DISRUPTED MOLECULAR PROCESSES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Abstract:
Introduction: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western countries. Due to dynamic clonal changes in a leukemic cell population over time, CLL is characterized by a highly variable clinical course. The expansion of subclones with different gene mutations, many of which are non-recurrent, makes CLL challenging to treat. We aimed to describe the clonal evolution in CLL, focusing on specific treatments and different stages of the disease. As defects in the *TP53* gene are strong predictive and prognostic markers in CLL and drive chemoimmunotherapy resistance, we particularly studied factors influencing the clonal development of *TP53*-mutated subclones.
Methods: We investigated a cohort of 62 CLL patients with detailed clinical characterization and known patterns of *TP53* mutation evolution. Whole-exome sequencing was employed to identify somatic variants at two to six different timepoints during the disease course of each patient. Subsequently, we analysed abnormal molecular pathways in defined patient groups and tracked their changes over time. To explore not only mutated components of the respective pathways but also pathway activities, we performed bulk RNA-seq on paired samples from a subset of 30 patients.
Results: Our analysis revealed striking competition among co-existing CLL subclones of individual patients. Mutations within recurrently affected genes exhibited either mutual exclusivity (e.g., in *TP53* and *BIRC3*) or co-occurrence (in *NOTCH1* and *ZMYM3*) within the same CLL subclones. We observed mutations in known CLL-associated genes but also novel unique mutations in other genes that emerged or diminished in defined patient groups under the treatment pressure and/or in relation to the clonal development of *TP53* mutations. Similar observations were made for disrupted molecular pathways. In timepoints where *TP53* mutations expanded rapidly, we found impaired Wnt and EGFR signalling. Interestingly, patients with late

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or no *TP53* mutation expansion exhibited detects in pathways requiring p53 activity, such as apoptosis, DNA damage checkpoints or Notch signalling.
Conclusion: Our study provides insights into the molecular aspects underlying clonal evolution in CLL, such as genetic variants and their interactions, gene expression patterns, and their changes. We observed progressive evolution of specific gene mutations, highlighting both their mutual exclusivity and co-occurrence within CLL subclones. Moreover, we observed several impaired pathways associated with different disease stages, treatment outcomes, and *TP53* status. These findings enhance our understanding of distinct disease phenotypes and suggest potential targets for personalized treatment strategies in CLL.

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Keywords: CLL, clonal evolution, mutation

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UNVEILING THE MICROBES OF PINOT BLANC FERMENTATION THROUGH METAGENOMICS

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Abstract: Microorganisms play a crucial role during winemaking, and understanding which microbes are present and active might help to minimise the spoilage of wine and improve on desired flavours. Microbial composition and activity were observed under controlled conditions in preparation of Pinot blanc ("Rulandské biele") from one vineyard during the years 2018, 2019, and 2020. Both fungi and bacteria are important for primary fermentation and malto-lactic fermentation, therefore total DNA and total RNA were isolated and genes for 16S and 28S rRNA were amplified to determine both bacterial and yeast profiles. Three phases of wine production were selected for testing. We tested inoculated must 2-3 days post inoculation, actively fermenting must and finally – young wine before filtration. We experimented on 3 batches with addition of selected strains of *Lachancea thermotolerans*, and *Metschnikowia pulcherrima*. The most apparent was seasonality and the effect of weather at given year. Naturally occurring yeasts *Starmerella* and *Hanseniaspora* were detected alongside major players like *S. cerevisiae*. The most dominant bacterial genera were *Gluconobacter*, *Komagataeibacter* and *Acetobacter*. We were able to detect contaminating coliform bacteria as a result of unexpectedly warm and humid conditions during the harvest in 2018. This method even detected plant pathogens *Penicillium*, *Botrytis*, and *Alternaria* in some samples, and might be indicative of the health of a vineyard. Selection of biomarkers for mitigation of problematic batches or pinpoint which strains to supplement in wine production are advantages of microbial profiling. This technique is ideal for controlled production and for spontaneous fermentation that's becoming a trend in some wineries. Applied metagenomics can improve the description of the biotic factor of terroir and offers data-driven decisions in wine production.

Keywords: Pinot blanc, grape must, fermentation, microbiome, spoilage organisms

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WHEN THE OLD BECOMES NEW INVESTIGATION OF THE MODE OF ACTION OF HYBRID COMPOUNDS DERIVED FROM ALREADY APPROVED ANTITUBERCULOTICS.

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Abstract: Tuberculosis (TB) is an infectious disease caused in humans by a bacillus *Mycobacterium tuberculosis* (*Mtb*). It typically affects lungs, but TB bacteria can also attack other parts of the body. The treatment of active TB recommended by the World Health Organisation takes 4 – 6 months using combination of multiple antibiotics, first-line antituberculosis drugs. However, the treatment is often prolonged and complicated due to the emergence of drug resistant *Mtb,* what requires the use of second-line drugs that are difficult to procure, and are much more toxic and less effective.

As shorter, better tolerated and more effective treatment is needed, it is necessary to search for new potent antibiotics. Nowadays, several strategies and efforts in the development of new antimicrobial agents are used, including the structure-based design of inhibitors for a single target through computational methods, the screening of commercial vendor libraries to identify compounds effective against mycobacteria, or derivatisation and combining of fragments of already approved drugs or clinical candidates. An indispensable part of this process is verification or revealing the specific molecular target and mechanism of action for a new drug.

Here, we report the elucidation of the mode of action of hybrid compounds combining the structures of second-line prodrug *para*-aminosalicylic acid (PAS) and first-line antitubercular drugs pyrazinamide, or derivative of isoniazid.

It was shown that in mycobacterial cells PAS is metabolized by dihydropteroate synthase and dihydrofolate synthase to hydroxyl-dihydropteroate and further to hydroxyl-dihydrofolate (Zheng *et al*., 2013). This antimetabolite inhibits essential enzyme, the dihydrofolate reductase and thus blocks the folate pathway.

Inhibition of folate pathway leads to perturbation of the activated methyl cycle and negatively affects the methionine and S-adenosylmethionine (SAM) levels in the cells (Nixon *et al*., 2014). SAM contributes to many cellular processes, including the formation of several functional groups in mycobacterial long chain fatty acids, mycolic acids.

To address the question of mechanism of action of tested compounds, we determined the sensitivity of *Mtb* overproducing dihydrofolate reductases DfrA or RibD to these compounds, tested the ability of methionine to complement their inhibitory effect, and analysed the lipid profiles of *Mtb* cells treated with each of the compound.

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Keywords: tuberculosis, mycobacterium, hybrid compounds, *para*-aminosalicylic acid

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POSITION OF WOMEN IN LIFE SCIENCES IN SLOVAK RESEARCH PROJECTS

DANICA ZENDULKOVÁ[1], GABRIELA GAVURNÍKOVÁ[1], ANNA KRIVJANSKÁ [1], ANDREA PUTALOVÁ[1]

1 Slovak Centre of Scientific and Technical Information

The Slovak Centre of Scientific and Technical Information (CVTI SR), as the national information centre for science, technology, innovation, and education of the Slovak Republic incorporates the perspective of gender equality into the content of science, research, and innovation. CVTI SR within its competence manages several national registers, which process scientific and research data and serve for their support. The poster presents a case study to examine the possibilities of processing data on the representation of women in science and research from data collected in Slovakia. The methodology consists of three steps. The first step is the identification of sources of sex-disaggregated data from the field of science and research in the Slovak Republic. Then follows the examination of the state of the art of tracking data in the identified data sources. The analysis of available data and the processing of the results is the next step. The Slovak Information System for Research, Development, and Innovation (SK CRIS), ensures the collection, processing, provision, and use of data from research, development, and innovation supported by public funds. SK CRIS contains a national register of research and development projects, a register of researchers, a register of organisations, information on research results and laboratory infrastructure. Incorporation of the CERIF data format within the SK CRIS enabled the collection of gender data. The demand for gender equality and equal opportunities for all apply for research teams at all levels. Our analysis relates to the registered research teams in the SK CRIS, i.e., is based on the links of researchers to projects, within the projects register. For the analysis of gender equality in life sciences, the data collection Projects implemented in 2021 was examined. Research projects registered in the SK CRIS information system are categorised by research area. As part of the categorization of the focus of the projects, it was necessary to identify those categories that belong to life sciences. The analysis shows that the share of women involved in research projects in general (regardless of the scientific area) in the monitored period was more than 45%. However, if we look at the share of women according to the basic groups of R&D fields, the representation of women begins to differ depending on the selected group of scientific fields. The share of women in biological sciences roughly corresponds to the share of women in medical sciences and in veterinary sciences and it is the highest among the monitored categories (approx. 62-63%). We can conclude that the involvement of women in research activities in the field of life sciences in Slovakia is above average. We consider this result to be a contribution to the creation and parameterization of science policy, including the principles of gender equality.

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Keywords: life sciences, research projects, women in science, data analysis

POSTER PRESENTATONS

1. **S. ADAMOVÁ, K. STRÁNSKÁ, J. SVATOŇ, M. BOHÚNOVÁ, E. ONDROUŠKOVÁ, M. JAROŠOVÁ, K. ZÁVACKÁ, K., J. KOTAŠKOVÁ, K. PLEVOVÁ (Brno, Czech Republic)**
   Application of long-read sequencing for detection of complex chromosomal rearrangements.

2. **K. ALFÖLDIOVÁ, M. DOLNÍK1, E. STRUHÁRŇANSKÁ, Z. LEVARSKI, S. STUCHLÍK (Bratislava, Slovak Republic)**
   Effect of bacterial growth rate on overproduction of Lon protease.

3. **H. AMSALU, F. BATISTA, AGNIESZKA ROBASZKIEWICZ, K. TAR (Debrecen, Hungary; Lodz, Poland)**
   Investigation of the role of PA200 independent of proteasome binding and activation.

4. **M. ANDREZÁL, S. ELNWRANI, A. BURDOVÁ, Z. REŠKOVÁ, J. KOREŇOVÁ, T. KUCHTA, H. DRAHOVSKÁ (Bratislava, Slovak Republic)**
   Analysis of bacteria and bacteriophages in whey from Slovakian bryndza cheese production.

5. **B. BAĎUROVÁ, R. ZÁHUMENSKÁ, T. PAVLIŠOVÁ, V. KUCHÁRIKOVÁ, M. JANÍČKOVÁ, S. NOVÁKOVÁ, E. HALAŠOVÁ, H. ŠKOVIEROVÁ (Martin, Slovak Republic)**
   Formation of dental stem cell 3d organoids and optimization their growth conditions for bone regeneration.

6. **M. BARANČÍK, M. BARTEKOVÁ, K. FERENCZYOVÁ, B. BOŤANSKÁ (Bratislava, Slovak Republic)**
   The role of marix metalloproteinases at pathological situations.

7. **K. BENEDIKOVÁ, I. SVERÁKOVÁ, V. YURCHENKO, A. HORVÁTH (Bratislava, Slovak Republic; Ostrava, Czech Republic)**
   Mitochondrial metabolism of monoxenous Trypanosomatids.

8. **A. BERTOVA, S. KONTAR, Z. SULOVA, A. BREIER, D. IMRICHOVA (Bratislava, Slovak Republic)**
   Cell death upon repeated administration of sulforaphane to P-glycoprotein negative or positive L1210 cell variants.

9. **M. BIELIKOVÁ, A. DUDÁŠ, E. HRČKOVÁ TURŇOVÁ3, Z. MAJCHRÁKOVÁ, J. TURŇA (Bratislava, Slovak Republic)**
   Methods for the detection of causal mutations causing canine cardiomyopathy to obtain individuals suitable as models of human cardiomyopathy.

10. **A. BIRKOVÁ, B. HUBKOVÁ, B. ČIŽMÁROVÁ, V. TOMEČKOVÁ, M. MAREKOVÁ (Košice, Slovak Republic)**
    Case reports in medical biochemistry course.

11. **N. BORZOVA, O. GUDZENKO, L. VARBANETS (Ukraine)**
    Fungal glycosidases for biotransformation of flavonoids and galactooligosaccharides.

12. **T. BURÁŇOVÁ, E. PAULENOVÁ, M. WIMMEROVÁ (Brno, Czech Republic)**
Novel two-domain lectin from bacterium *Photorhabdus laumondii.*

13. **A. BURDOVÁ, A. BUGALOVÁ, J. BUGALA, M. ANDREZÁL, E. KACLÍKOVÁ, J. MINAROVIČOVÁ, A. VÉGHOVÁ, J. KOREŇOVÁ, H. DRAHOVSKÁ (Bratislava, Slovak Republic)**
Whole genome analysis of *Listeria monocytogenes* strains isolated from food processing environments.

14. **K. ČERNOVSKÁ, J.P PORC, K. ZÁVACKÁ, E. ONDROUŠKOVÁ, M., Š POSPÍŠILOVÁ, K. PLEVOVÁ K (Brno, Czech Republic)**
Chromatin conformation analysis reveals genome structural and functional complexity in chronic lymphocytic leukemia.

15. **L. ČUCHRÁČ, M. MYDLÁROVÁ BLAŠČÁKOVÁ, J. FIRMENT, J. ŠIMONOVÁ, J. BERNASOVSKÁ, J. VAŠKOVÁ, A. BUKOVÁ (Košice, Slovak Republic; Prešov, Slovak Republic)**
Plasma cholinesterase polymorphisms monitoring important either for the choice of muscle relaxant or for the blockade reversal.

16. **M. DABIRI, Z. LEVARSKI, J. TURNA, S. STUCHLIK (Bratislava, Slovak Republic)**
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17. **K. DIBDIAKOVÁ, A. EVINOVÁ, E. BARANOVIČOVÁ, P. RAČAY, R. PÉČOVÁ, M. POKUSA (Martin, Slovak Republic)**
Effect of ATP-dependent potassium channels on mitochondrial dynamics in an *in vitro* model of neurodegeneration.

18. **J. DLAPOVÁ, K. HRIŇOVÁ, Ľ. KORMANOVÁ, E. STRUHÁRŇANSKÁ, Z. LEVARSKI, J. TURŇA, S. STUCHLÍK (Bratislava, Slovak Republic)**
Production of mutant Taq DNA polymerase in *Vibrio natriegens* expression system.

19. **M. DOLNÍK, E. STRUHÁRŇANSKÁ, S. STUCHLÍK (Bratislava, Slovak Republic)**
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20. **Z. DOSTÁL, H. PŘICHYSTALOVÁ, M. MODRIANSKÝ (Olomouc, Czech Republic)**
2,3-dehydrosilybin protects cardiomyoblasts against hypertonic cytotoxicity in temperature dependent manner.

21. **A. DUSÍKOVÁ, L. LUKÁČOVÁ, S. HOUDEKOVÁ, B. TULIPÁNOVÁ, E. MOKROŠ, J. KRAHULEC (Bratislava, Slovak Republic)**
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22. **S. ELNWRANI, B. MARKUSKOVÁ, M. ANDREZÁL, H. DRAHOVSKÁ (Bratislava, Slovak Republic)**
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23. **EVINOVÁ, K. DIBDIAKOVÁ, R. PÉČOVÁ, P. RAČAY, M. POKUSA (Martin, Slovak Republic)**
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24. **V. FABIANOVÁ, S. HUSZÁR (Bratislava, Slovak Republic)**
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25. **V. FABIANOVÁ, K. SAVKOVÁ, S. HUSZÁR, K. MIKUŠOVÁ (Bratislava, Slovak Republic)**
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26. **L. FECKOVA, R. JAVOROVA, B. REZUCHOVA, D. CSOLLEIOVA, R. NOVAKOVA, F. OPATERNY, J. KORMANEC (Bratislava, Slovak Republic)**
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27. **V. FEDOROVA, T. BIRO, K. SIPOSOVA, M. HUMENIK (Košice, Slovak Republic; Bayreuth, Germany)**
Self-assembling of spider silk protein conjugated with biologically active DNA-aptamers.

28. **A. FICEK, V. MEDOVÁ VERONIKA, Ľ. KÁDAŠI, A. ŠOLTÝSOVÁ (Bratislava, Slovak Republic)**
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29. **G. FLORES–RAMÍREZ, K. PALKOVIČOVÁ, K. MERTENS, G. BEKE, M. DANCHENKO, G. MITULOVIC5, Ľ. ŠKULTÉTY (Bratislava, Slovak Republic**; **Jena, Germany**; **Wien, Austria**; **Prague, Czech Republic**)
Infection of THP-1 derived macrophages by *Coxiella burnetii* bacterium.

30. **G. FLORES-RAMÍREZ, F. ZÚÑIGA-NAVARRETE, H. KABICKOVA, J. DRESLER, Ľ. ŠKULTÉTY (Bratislava, Slovak Republic**; **Prague, Czech Republic**)
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31. **M. GHANI, M. ALKHATIBE, B. SZABÓ, L. VARGA, K. VADÁSZI, K. TAR (Debrecen, Hungary)**
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32. **M. GRMAN, J. ŠTEPANOVSKÝ, K. ONDÁČOVÁ, Z. ŠEVČÍKOVÁ TOMÁŠKOVÁ ZUZANA (Bratislava, Slovak Republic)**
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34. **J. GUŽIKOVÁ, M. LÍŠKOVÁ, Ľ. HUDÁK, M. BRODŇANOVÁ, P. RAČAY (Martin, Slovak Republic)**
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35. **J. HATOK, Z. MAJERČÍKOVÁ, K. DIBDIAKOVÁ, T. GALANDA, R. RICHTEROVÁ, R. MURÍN, P. RAČAY (Martin, Slovak Republic; Banská Bystrica, Slovakia)**
Relationship between expression of matrix metalloproteinases and their tissue inhibitors in patients with brain tumour.

36. **Z. HATOKOVÁ, A. EVINOVÁ, R. ZÁHUMENSKÁ, E. HALAŠOVÁ, S. NOVÁKOVÁ, H. ŠKOVIEROVÁ (Martin, Slovak Republic)**
Comparative analysis of human mesenchymal stem cells derived from various sources and human fibroblasts.

37. **L. HEJDUK, M. STRNAD, M. BAUMGARTNER, F. DYČKA, L. GRUBHOFFER, J. ŠTĚRBA, R.O.M. REGO, N. MÜLLER, A. RATHNER (České Budějovice, Czech Republic; Linz, Austria)**
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38. **E. HOLUB, G. PAPP, H.L. PÁLINKÁS, B.G. VÉRTESSY, A. BÉKÉSI (Budapest, Hungary)**
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39. **K. HRIŇOVÁ, J. DLAPOVÁ, Ľ. KORMANOVÁ, E. STRUHÁRŇANSKÁ, Z. LEVARSKI, J. TURŇA, S. STUCHLÍK (Bratislava, Slovak Republic)**
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40. **Ľ. HUDÁK, M. BRODŇANOVÁ, J. GÚŽIKOVÁ, M. LÍŠKOVÁ, P. RAČAY (Martin, Slovak Republic)**
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46. **K. JIRASKOVA, K. POSTULKOVA, R. ANDRÝS, L. ZEMANOVA, K. BOUSOVA, J. VONDRASEK (Prague, Czech Republic; Hradec Kralove, Czech Republic)**
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47. **B. KASCAKOVA, J.P.K. BRAVO, L. ASPINALL, R.F. THOMPSON, A. BORODAVKA, I. KUTA SMATANOVA, R. (Ceske Budejovice, Czech Republic; Cambridge, UK; Leeds , UK; Munich, Germany; Luebeck, Germany)**
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49. **J. KORMANEC, F. OPATERNY, L. FECKOVA, R. JAVOROVA, B. REZUCHOVA, D. CSOLLEIOVA, R. NOVAKOVA (Bratislava, Slovak Republic)**
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50. **D. KORNIEIEVA, B. KALOČAYOVÁ, D. ŠNÚRIKOVÁ, K. FERENCZYOVÁ, M. BARTEKOVÁ, Š ZORAD, J. VLKOVIČOVÁ (Bratislava, Slovak Republic)**
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51. **V. KUCHÁRIKOVÁ, T. PAVLIŠOVÁ, R. ZÁHUMENSKÁ, J. STRNÁDEL, H. ŠKOVIEROVÁ, E. HALAŠOVÁ (Martin, Slovakia)**
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52. **V. KULDA, J. POLIVKA, M. SVATON, T. VANECEK, M. BURESOVA, K. HOUFKOVA, B. VANKOVA, M. PESTA (Pilsen, Czech Republic)**
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54. **L. LEVARSKÁ, Z. LEVARSKI, S. VOŠKO, Ľ. KORMANOVÁ, G. GAVURNÍKOVÁ, J. TURŇA, S. STUCHLÍK (Bratislava, Slovak Republic)**
Production of DEK1 LG3 domain in *Vibrio natriegens.*

55. **Z. LEVARSKI, L. LEVARSKÁ, J. ŠARKANOVÁ, E. STRUHÁRŇANSKÁ, G. GAVURNÍKOVÁ, J. TURŇA, S. STUCHLÍK (Bratislava, Slovak Republic)**
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56. **M. LÍŠKOVÁ, A. EVINOVÁ, J. GUŽIKOVÁ, Z. TATARKOVÁ, M. BRODŇANOVÁ, Ľ. HUDÁK, P. RAČAY (Martin, Slovak Republic)**
The effect of TUDCA and PBA on mitochondria in SH-SY5Y cells.

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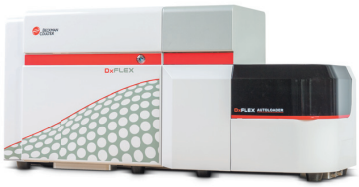

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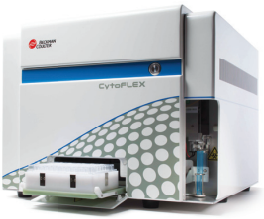

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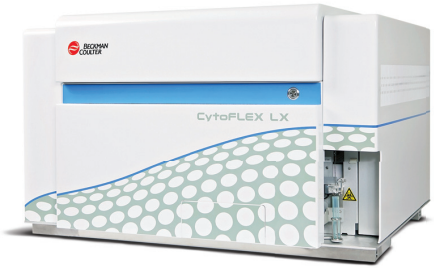

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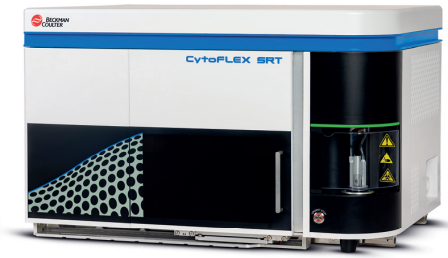

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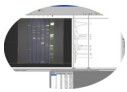

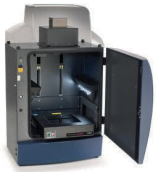

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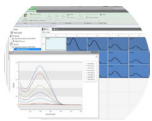

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