THE PROGRAMME AND ABSTRACT BOOK

LITHUANIAN BIOCHEMICAL SOCIETY
2022 MINI-CONFERENCE:
BIOCHEMISTRY IN THE BIG DATA AGE

VILNIUS, SEPTEMBER 30, 2022

Welcome word

Dear Colleagues, Members of the Lithuanian Biochemical Society, dear Guests,

On behalf of conference organizing committee, I would like to welcome you to Lithuanian Biochemical Society 2022 Mini-Conference, entitled “Biochemistry in the Big Data Age”. It is of special honor to organize such an awaited meeting in these turbulent times. Creativity and passion are the answers to any challenge, so is the never-ending progress of Biochemistry. Let us be confident in recognition of excellence. I wish you a very best experience in this Venue, short by time albeit full of revelations!

Saulius Serva
Secretary of LBS

Organizing Committee of the conference

Prof. Saulius Serva	Dr. Valeryia Mikalayeva
Dr. Inga Songailiene	Prof. Vilmantė Borutaitė
Dr. Dukas Jurėnas	Dr. Vytautas Smirnovas

Acknowledgement

PROGRAMME OVERVIEW

10:00 – 10.10 Opening (dr. D. Matulis, Chair of Lithuanian Biochemical Society)

10.10 Virginijus Šikšnys „Being a part of European Life science community"

10.30 Stephen Knox Jones Jr. „Next-generation biochemistry applications in gene editing"

11.00 Urtė Neniškytė "Editing the brain: application of emerging molecular tools to alleviate neurological diseases"

11.30 Aleksej Železniak „Bridging machine intelligence and synthetic biology for understanding and engineering of biological systems"

12.00 Artūras Petronis „Chrono-epigenomics of human disease"

12.30 – 13.30 Lunch

13.30 Justas Dapkūnas „Protein structure modeling using AlphaFold: what works and what does not"

13.50 Irmantas Rokaitis „Accelerating enzyme engineering with artificial intelligence"

14.10 Saulius Gražulis „The Importance of Having Data"

14.30 – 15.00 Coffee break

15.00 Gytis Dudas „Small victories of the Lithuanian SARS-CoV-2 genomic surveillance programme"

15.20 Rapolas Žilionis „Droplets as a tool for high-throughput single-cell analysis"

15.40 Vida Mildažienė „STEAM ugdymas ir jo situacija Lietuvoje"

16.00 – 16.30 LBS members general meeting

16.30 – 18.00 Poster session

18.30 – 18:45 Closing ceremony

POSTER ABSTRACTS

1. ACTIVITY OF TYPE III CRISPR-CAS ASSOCIATED RING NUCLEASE

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Type III CRISPR-Cas systems employ Csm or Cmr ribonucleoprotein complexes to cleave RNA [1] and DNA [2] to confer immunity against viruses and foreign plasmids. After recognition of target RNA Csm/Cmr also synthesizes 3-6 nt cyclic oligoadenylates (cOA) from ATP [3]. cA_4 or cA_6 allosterically activates CARF domain-containing accessory proteins. Bioinformatic analysis revealed that in prokaryotic genomes CARF domain could be fused with different putative DNases, RNases, adenosine deaminase and other domains [4]. Together with Csm/Cmr these auxiliary effectors provide immunity to the host. However, they could be toxic to a cell and result a dormancy or an altruistic cell death. To avoid host damage, the cOA concentration is tightly regulated by (i) cOA synthesis that is controlled through target RNA degradation by Csm [2], (ii) cOA degradation by specialized enzymes called ring nucleases [5].
Several uncharacterized CRISPR-associated genes were hypothesized to possess ring nuclease activity [4]. In this study we present results to demonstate that Csx20 family proteins act as ring nucleases *in vitro* and *in vivo*.

References

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2. MECHANICAL PROPERTIES OF CASCADE-CAS3 USING SINGLE-MOLECULE MAGNETIC TWEEZERS

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CRISPR is an antiviral defence system in bacteria and archaea. These systems require nucleoprotein complexes to detect and neutralise viral DNA. Cascade-Cas3 functional complex is found in CRISPR type I systems and works as a DNA shredder. A multisubunit DNA targeting ribonucleoprotein Cascade enables accessory helicase-nuclease protein Cas3 to cut foreign DNA within a cell [1]. Knowledge about the mechanism of this complex would present us with a genome editing tool which would be suitable for performing large deletions. However, the exact mechanism of DNA unwinding and degradation is still unknown. Cas3 acts as a motor protein and requires ATP to generate mechanical work. Therefore, understanding the mechanical properties is crucial to elucidating the mechanism. Thus, the aim of this research is to characterise the mechanical properties of Cascade-Cas3.

Here we are using a single-molecule approach, namely magnetic tweezers, to elucidate the mechanism of action of Cascade-Cas3. Magnetic tweezers allow manipulation of single DNA molecules attached to paramagnetic beads. Applying this technology enables us to observe protein-DNA interactions in real time and perturb it, making it a suitable method to examine the mechanical properties of helicase and nuclease activities of Cascade-Cas3.

References

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3. STAT3 SIGNALING PATHWAY AS A POTENTIAL THERAPEUTIC TARGET IN TRIPLE NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer and accounts for much higher recurrence and metastasis rates. TNBCs are a specific subtype of epithelial breast tumours that are immunohistochemically negative. TNBC is the most lethal subtype of breast cancer owing to its high heterogeneity, aggressive nature, and lack of treatment options[1].
Currently, chemotherapy remains the standard of care for TNBC treatment due to the absence of molecular targets, but unfortunately, patients frequently develop resistance which underscores the need for developing novel therapeutic and preventive approaches for this disease[2]. It has become evident that the development of TNBC chemoresistance is multifaceted and based on the elaborate interplay of the tumor microenvironment. Alterations of multiple signaling pathways govern these interactions[1]. Recent evidence from clinical trials and preclinical studies have demonstrated a pivotal role of the STAT3 (signal transducer and activator of transcription 3) signaling pathway in the initiation, progression, and metastasis of TNBC[2].
The aim of this study is to investigate the expression of *STAT3*, *ALDH1A1*, *NFIB* genes, compare it with samples before and after treatment and discuss their potential as biomarkers for therapeutic purposes.
In this study, we used RT-qPCR to determine genes expression in paired 53 blood plasma samples of TNBC patients before and after treatment. The results revealed statistically significant differences in gene expression of *STAT3* ($p=0.0016$) between samples before and after treatment. Furthermore, using logistic regression we determined that these three genetic biomarkers have potential to distinguish clinically significant samples before treatment from after treatment, however, more samples need to be tested to confirm the validity of the model.
In conclusion, the studied genes, especially the *STAT3* oncogene, are promising targets, which may be used for further TNBC research, prevention, and therapy.

References

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4. APPLICATION OF LONG PROKARYOTIC ARGONAUTE PROTEINS TO CUT DOUBLE-STRANDED DNA

Viktorija Rainytė[1], Paulius Toliušis[1], Mindaugas Zaremba[1]

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Prokaryotes are the most abundant living organisms in our planet. In order to survive from constantly invading viruses (phages), prokaryotes evolved various antiphage defense systems such as restriction-modification and CRISPR-Cas systems. Through time, scientists are discovering more and more new antiviral defense systems, one of which is the prokaryotic Argonaute proteins (pAgos). pAgos are divided into two groups: catalytically active long pAgos and inactive short pAgos [1]. However, long pAgos proteins possess poor catalytic activity on double stranded DNA at physiological temperatures (~37°C), preventing them from potential application in genome editing [2, 3]. Efficient double-stranded DNA hydrolysis requires unwinding of the DNA duplex, where individual DNA strands can be cut by pAgos loaded with specific short guide DNAs. One of the long catalytically active prokaryotic Argonaute proteins is CbAgo from *Clostridium butyricum*. The CbAgo protein has been extensively studied because it is active at physiological temperatures, but the present knowledge is that double-stranded DNA cutting is complicated and inefficient, particularly in GC-rich regions. Here we present 3 strategies that could help CbAgo proteins efficiently cut double-stranded DNA using additional proteins such as SpCas9 (*Streptococcus pyogenes*), StCascade (*Streptococcus thermophilus*) or SpCas9 with RepX (*Escherichia coli*).

References

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5. DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST B-LACTAMASES CAUSING ANTIMICROBIAL RESISTANCE

K. Juškaitė, R. Petraitytė-Burneikienė, A. Žvirblienė

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According to World Health Organization (WHO) antimicrobial resistance (AMR) is one of the greatest threats to global health in this century. Currently 700 000 deaths are linked to AMR each year globally. It is estimated that 10 million lives a year may be lost due to AMR by 2050. Misuse of antimicrobials is the main driver in the development of new AMR mechanisms and difficulties in treating common infectious diseases. Therefore, development of reliable and rapid diagnostic tools is a priority in the context of AMR. In the healthcare system, about 60% of prescribed antibiotics consist of β-lactams. Therefore, bacterial β-lactamases that degrade β-lactam antibiotics can be used as potential targets for diagnostics.

The aim of this study was to develop β-lactamase specific monoclonal antibodies (MAbs) to use them as molecular tools for diagnostic purposes in various immunoassays, such as multiplex ELISA, lateral flow or automated fluorescent bead-based immunometric assays. These immunoassays are rapid, simple to perform and highly promising for point-of care diagnostics. Considering the WHO list of antibiotic resistant priority pathogens, we have selected four β-lactamases – ACT-14, NDM-1, PDC-195 and CMY-34 – as targets for MAb development. Recombinant antigens have been produced in *Escherichia coli* expression system and purified using affinity chromatography. Large collections of mouse MAbs against each target have been generated by hybridoma technology (60 MAbs in total). The specificity and cross-reactivity of the MAbs have been studied by different immunoassays including a competitive ELISA and Western blot. The most promising pairs of MAbs have been selected to develop sandwich ELISA for a quantitative detection of each target. The assay has been optimized to achieve the highest sensitivity and specificity. Currently the optimized sandwich ELISA systems are being tested with biological samples containing resistant bacteria and natural β-lactamases. The next step of MAb application is development of multiplex quantitative tests for 4 targets in one sample. We believe that well-characterized MAbs against β-lactamases have high diagnostic potential.

6. ISOLATION, IDENTIFICATION AND INVESTIGATION OF MICROORGANISMS GROWING ON COFFEE

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Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid found in coffee, tea[1]. The major catabolic pathway of caffeine is: caffeine → theobromine/theophylline → 3-methylxanthine → xanthine → uric acid → allantoin → allantoic acid → CO_2 + NH_3 [2]. It is known that microorganisms such as fungi and bacteria can grow on the coffee bean fruit. Bacteria are able to use caffeine as a sole carbon and nitrogen source. There are two bacterial mechanisms for caffeine degradation: *N*-demethylation and C-8 oxidation[3].

In this study, two types of fungi (*Geotrichum silvicola* and *Penicillium* spp.) and eight types of bacteria, some of them are *Klebsiella* species were investigated. These microorganisms were isolated from coffee bean grounds or instant coffee, and identified by DNA sequencing. Minimal media with caffeine was used to determine the growth of microorganisms in a caffeine environment. The ability of bacteria to degrade caffeine was investigated by high-performance liquid chromatography (HPLC) to identify newly formed caffeine metabolites.

References

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7. CRYSTAL STRUCTURES OF TIR-DOMAIN PROTEINS FROM THOERIS BACTERIAL ANTIVIRAL SYSTEM

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Thoeris is a bacterial antiviral defense system composed of two genes thsA and thsB [1]. ThsB protein contains Toll/interleukin-1 receptor (TIR) domain which is a canonical component of animal and plant immune systems. Based on domain oraganization of ThsA proteins, Thoeris systems can be subdivided into two types: type I ThsA is composed of a sirtuin-like (SIR2) and STALD domains, type II ThsA is composed of a transmembrane and Macro domains. Representative structures of the type I ThsA and ThsB proteins and their functional studies were published recently [2,3]. Type I ThsB protein recognizes phage infection and produces a signaling molecule variant of cyclic ADP-ribose (v-cADPR). v-cADPR is then bound by ThsA STALD domain and activates NAD hydrolysis by SIR2 domain resulting in NAD depletion and the host cell death [3]. In plants, intracellular pathogen sensing by immune receptors also triggers their TIR domains to generate v-cADPR. Type II ThsB presumably produces different unknown signaling molecule which could be recognized by type II ThsA Macro domain. Here we present crystal structures of two ThsB proteins from type II Thoeris systems. In vivo data show that functional ThsB is required for antiphage activity of the Thoeris system. The ThsB structures show similarities with nucleoside ribosyltransferases, eukaryotic TIR domains and ThsB from the type I Thoeris system. Differently from the most TIR domain proteins, ThsB protein is a monomer, however studies of eukaryotic TIR-domain proteins suggest that oligomerization may be required for ThsB activity.

References

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8. GENERATION OF IMMUNOLOGICAL TOOLS TO INVESTIGATE *GARDNERELLA* SPP. ADHESIVE PROTEINS

R. Reškevičiūtė[1], A. Bulavaitė[2], I. Dalgėdienė[1], M. Plečkaitytė[1], A. Žvirblienė[1]

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Bacterial vaginosis (BV) is one of the most common vaginal infections associated with impaired vaginal microflora that affects women of childbearing age. BV is associated with various adverse outcomes not only relating to women's fertility, reproductive health, or pregnancy, but also to women's mental health [1]. *Gardnerella* spp. is the most associated bacterium with BV and has been detected in almost all vaginal samples from women suffering from BV [2].
Adhesins are cell-surface components that are responsible for the recognition of host cells and adhering to them. Studies on other bacteria show that various adhesins are proven virulence factors that are significant in bacteria's pathogenesis [3, 4]. However, the adhesins of bacterium *Gardnerella* spp. are very little studied and described.
Gardnerella spp. collagen-binding protein (CNA) and M protein repeat protein (MPR) are surface-exposed proteins. Bioinformatic analysis revealed certain motifs of CNA and MPR that show their potential of providing bacteria with adhesion to host tissues.
Molecular tools – high affinity and specificity monoclonal antibodies (MAbs) specific to recombinant CNA and recombinant MPR protein were generated using hybridoma technology. Both collections of anti-CNA and anti-MPR MAbs were used to investigate the interaction between CNA or MPR and extracellular matrix proteins collagen type I, III, and IV, fibronectin, and fibrinogen. Indirect ELISA method revealed specific and dose dependent interaction between CNA and fibrinogen and between MPR and fibronectin, fibrinogen. Additionally, an interaction between CNA or MPR and ME-180 cervical epithelial cells was evaluated using flow cytometry. We were able to demonstrate the dose dependent interaction between MPR and ME-180 cells. However, no signal was detected in the case of CNA and ME-180 cells.
We have demonstrated that generated and characterised anti-MPR and anti-CNA MAbs are suitable tools in MPR and CNA studies. Thus, they will be further used in both, MPR and CNA functional research to understand the molecular mechanisms of *Gardnerella* spp. bacteria pathogenicity.

References

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9. ENHANCEMENT OF STEVIA SWEETNESS BY COLD PLASMA

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Short seed treatment with non-thermal or cold plasma (CP) is well-known stressor which stimulates seed germination, grown plant morphometric parameters, biomass production, and disease resistance in different plant species by inducing changes in plant biochemical phenotype. The activities of enzymes, the amounts and ratios of different secondary metabolites are markedly changed after some treatments, however, there are still not enough knowledge in molecular mechanisms to control and predict treatment effect.
Stevia rebaudiana Bert. (Bertoni) is cultivated abundantly in many countries as economically important source of natural low-calorie sweeteners, steviol glycosides (SGs). Beside the sweet taste, stevia extract and SGs are associated with antihypertensive, antihyperglycemic, antioxidant, anti-inflammatory, antifungal, antimicrobial activities, and anticariogenic action. Due to these various beneficial attributes and absence of side effects in long term use, stevia extracts and SGs are intensively investigated.
We have demonstrated the potential of CP to stimulate SGs biosynthesis in stevia by using different types of CP generation (dielectric barrier discharge (DBD) plasma and capacitively-coupled (CC) plasma) applied on stevia seeds for 2-7 min. The maximum effect obtained was 11-fold-increase in stevioside, most abundant SG in stevia, concentration[1)]. We have shown this stimulating effect is reproducible and manifests at different extent in different cultivars of stevia and using seeds of various storage time.
In contrast to CP-induced SGs production stimulation, the treatment has negative impact on the content of total phenolics (TPC), flavonoids (TFC) and antioxidant activity. This effect of CP differs from the effect of various abiotic physical and chemical stressors which usually simultaneously increase production of SGs and phenolic compounds.
It can be concluded that a short time pre-sowing treatment of seeds with CP can be a powerful tool for the enhancement of biosynthesis/accumulation of SGs in stevia plants.

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10. CHARACTERIZATION OF ENDOMETRIAL STROMAL CELLS IN 2D AND 3D SYSTEMS

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Infertility is a widespread disease of the human reproductive system when women cannot conceive after attempts lasting one year or more. The World Health Organization estimates that around 186 million individuals confront this health problem. In as many as 20 % of cases, no pathology is detected, but conceiving is unsuccessful.[1] As research at the molecular level could provide knowledge for diagnostics and treatment development, it becomes more crucial. Focus is given to the endometrium – a tissue of women reproductive system, participating in processes required for fertilized egg implantation.[2]

In this study, we characterized endometrial stromal cells from the conceived and unconceived patients according to the International Society for Cellular Therapy.[3] They showed differentiation potential towards adipogenic, osteogenic, and chondrogenic lineages. The cells were positive for typical mesenchymal stem cell surface markers such as CD90, CD73, CD105, and were negative for untypical markers such as CD34, CD31, CD45. Stem cells were cultivated in 2D (monolayer) and 3D (spheroid) systems, as the 3D system is structurally closer to native conditions. The diameter of formed spheroids from conceived patients' cells was 1.2-fold bigger than in unconceived group. In addition, we aimed to investigate how infertility and different growth conditions impact gene expression. The expression of genes linked with pluripotency (*SOX2, OCT4, NANOG*) increased in the 3D cell culture of conceived patient and remained unchanged in the unconceived patient. Genes related to hypoxia and apoptosis (*HIF1a, BAK1*), and cell cycle (*p21, CCNA2, CDK2, CCNE2*) were upregulated in unconceived patient cells. The expression of paracrine signaling-related genes (*VEGFA, FGF2, IL-8, IL-6*) was variable between conceived and unconceived patients, but increased in 3D culture conditions. In most cases, gene expression differed among 2D and 3D systems.

Overall, our results suggest that 3D cultures could be used in developing novel tools for infertility therapy and diagnostics, although further and more detailed studies are required.

References

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11. EXPLORING THE EFFECT OF AMYLOID-SPECIFIC MOLECULES ON PROTEIN AGGREGATION

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Aggregation of amyloid proteins and the resulting amyloid fibrils are associated with various neurodegenerative disorders, such as Alzheimer's, Parkinson's or prion diseases. Although this field has been under intense investigation for many years, only a few effective treatments or drugs have been discovered so far. Often, compounds show potential aggregation inhibitory efficacy during *in vitro* assays, but fail in clinical trial stages. The effectiveness of inhibitory molecules *in vitro* is usually analyzed using various fluorescent dyes, such as thioflavin-T (ThT) or 8-anilinonaphthalene-1-sulfonic acid (ANS) [1]. Despite their wide application, the conditions under which amyloid-specific dye molecules are used can vary quite significantly. Studies have shown that increasing the ionic strength of the solution increases the concentration of ThT molecules attached to the fibrils and affects their fluorescence intensity [2]. For this reason, we investigated the influence of ionic strength of the solution or different strains of insulin on the affinity of ANS. For this reason, we investigated how ANS binding and fluorescence parameters are affected by environmental conditions, as well as the surface of different insulin amyloid fibrils.

Different strains of insulin fibrils were obtained under 4 aggregation conditions. Human recombinant insulin powder was dissolved in 20% acetic acid solution, containing 100 mM NaCl (AC conditions), 100 mM phosphate (PH20 conditions), 100 mM phosphate, containing 100 mM NaCl (PH24 conditions) and 1x PBS (PH74 conditions) to a final protein concentration of 200 µM. The prepared AC, PH20 and PH74 samples were incubated at 60 °C for 24 hours without agitation. The PH74 samples were incubated at 60 °C under constant 600 RPM agitation, with two 3 mm glass beads present in the test tube. After aggregation, samples were centrifuged at 12 500 RPM for 10 min and resuspended in MilliQ water (for ionic strength assays) or 1x PBS buffer (for dye affinity assays) to a final fibril concentration of 400 µM. The resulting sample fluorescence intensity, absorbance and excitation-emission matrices were then analyzed.

The results of this work demonstrate that ANS molecules have a higher affinity towards insulin fibrils at low ionic strength conditions. In addition, we show that distinct insulin amyloid fibrils have specific ANS-binding affinities, as well as conformation-specific ANS fluorescence quantum yields.

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12. STUDY OF SHORT PROKARYOTIC ARGONAUTE- NUCLEIC ACIDS INTERACTIONS *IN VIVO*

Edvinas Jurgelaitis[1], **Evelina Zagorskaitė[1], Algirdas Grybauskas[1], Mindaugas Zaremba[1]**

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Restriction modification enzymes, CRISPR-Cas systems, and prokaryotic Argonaute proteins (pAgo) contribute to the defense against invading mobile genetic elements in bacteria. Unlike the first two systems which have been extensively studied, there is not a lot of data involving short prokaryotic protein structure, functions, and mechanism of action. Each unique pAgo protein can use different nucleic acid as a guide - RNA or DNA, sometimes both. Also, different pAgos show bias towards specific nucleotides. They can discern the first 1 or 2 nucleotides in guide sequence[1]. This would explain why pAgos use unique guides to target sequence-specific mobile genetic elements such as bacteriophages or plasmids[2].

Our research object – short pAgo proteins – makes up to 60% of all identified pAgo proteins. Here we focused on two short pAgos - RpTIR/Ago from *Rhodopseudomonas palustris* and XaMrr/Ago from *Xanthobacter autotrophicus* which have Toll-Interleukin Receptor (TIR) or Modified DNA rejection and restriction (Mrr) effector domains respectively. Our objective is to determine which nucleic acid – RNA or DNA – is bound to the protein in vivo and whether is there any nucleotide preference in that specific nucleic acid. This would help select the most optimal guide oligonucleotides for further research about protein structure, enzymatic activity, and mechanism of action.

Here lysis under mild conditions we show that in *E. coli* cells expressing RpTIR/Ago and XaMrr/Ago short pAgos are bound short RNAs. Using Illumina's next-generation sequencing we were able to determine which nucleotides were biased in each protein – RpTIR/Ago tended to bind dinucleotide 5′- AU, XaMrr/Ago had a distinct preference to use 5′- A as a first nucleotide.

These successful results are important for further research in vitro where short pAgos function and mechanism of action will be analyzed.

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13. CRYSTALLIZATION OF THSB PROTEINS FROM BACTERIAL ANTIVIRAL SYSTEM THOERIS

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Recently, bacterial antiviral systems have been shown to cluster side-by-side in bacterial genomes to form 'defense islands' leading to discovery of many new systems[1),2)]. One of these systems is called Thoeris[2)]. It is composed of two genes, *thsA* and *thsB*[2)]. ThsB shares homology with Toll-interleukin receptor (TIR) domains and may be present in several copies[2)]. Two types of ThsA are found: one containing SIR2 and SLOG domains, another composed of transmembrane and Macro domains[2)]. It was shown, that in the case of phage infection ThsB proteins produce signalling molecules from NAD[3)]. However, it is not clear how ThsBs recognize phage infection and how they are activated to synthesize the signalling molecule[3)]. Based on protein sequence ThsBs were classified into several clusters. To gain more insight into the function of the ThsB proteins, we decided to determine X-ray structures of ThsBs belonging to 3 different clusters. For this type of analysis, protein crystals are required, which are obtained by performing crystallization experiments of the purified recombinant protein. Crystal structures of the ThsB proteins would provide the basis for further studies on the recognition of bacteriophage infection and the regulation of the enzymatic activity of the ThsB proteins.

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14. CLONING, EXPRESSION AND PURIFICATION OF A PUTATIVE cOA-REGULATED ADENOSINE DEAMINASE

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Type III CRISPR-Cas systems adopt a unique signalling pathway for protection against invasive nucleic acids. Upon encounter foreign transcripts, the effector complex of type III systems starts synthesizing cyclic oligoadenylates (cOA) from ATP, which act as second messengers and trigger auxiliary effectors[1, 2]. These effectors are composed of a cOA-recognizing sensory CARF (CRISPR-associated Rossmann fold) domain fused to an effector domain. Effector domains can be of various types, such as ribonuclease, deoxyribonuclease, deaminase, protease, etc[3]. Currently, only auxiliary effectors with nuclease domains have been studied in detail, nevertheless, in order to elucidate the role of insufficiently researched signalling pathways in bacterial defence mechanisms, more attention needs to be paid to other effector domains. One such effector domain is adenosine deaminase (ADA).

Here we expressed and purified *Candidatus Cloacimonas acidaminovorans str. Evry* CARF domain containing protein fused to a putative ADA domain. According to the results of multi-angle light scattering coupled with size exclusion chromatography (SEC-MALS) analysis, the protein was found to be a dimer. Even though CARF domain of this protein has been hypothesized to exhibit ring nuclease activity[4], no cOA cleavage was observed. However, we identified the activating molecule by measuring changes in the melting point of the protein in the presence of different cOA.

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15. THE DEPENDENCE OF THE RESPONSE OF RADISH *(RAPHANUS SATIVUS L.)* TO IRRADIATION WITH COLD PLASMA ON SEED COLOR: CHANGES IN SPROUT GROWTH, CONTENT OF PHOTOSYNTHETIC PIGMENTS AND SECONDARY METABOLITES

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It has been shown that seed germination kinetics, content of phytohormones and antioxidants, as well as changes in seedling growth in response to atmospheric cold plasma (CP) treatment are dependent on the color of *Raphanus sativus* seeds [1]. This study aimed to estimate how the effects of CP treatment on the early growth as well as on the content of photosynthetic pigments and secondary metabolites in sprouts of two cultivars of *R. sativus* are dependent on the seed color. Seeds of 'Fujitaseed' and 'Nichinou' cultivars were separated by the color to grey and brown. Seeds were CP irradiated for 3 min using a scalable dielectric barrier discharge (DBD). The relationship between the seed color and germination, morphometric sprout parameters, antioxidant activity and metabolite levels were estimated.
Results showed that while most of the analyzed sprout parameters differed between grey and brown seeds, the ratio of parameters between the seed colors mostly depended on the cultivar. The color of the seed coat did not have impact on seed maximal germination (%), however germination half-life (Me) and germination uniformity (Qu) was different between grey and brown seeds, moreover, this difference was depended on the cultivar. Morphometric measurements showed that sprouts grown from brown seeds had longer (11-13%) and heavier (14-20%) shoots than sprouts grown from grey seeds, but root length and weight differences were more dependent on the cultivar. The antioxidant activity of the sprouts, the total amount of phenolic compounds, flavonoids and carotenoids also depended on the cultivar, but not on the color of the seed. However, sprouts of brown seeds had higher content of chlorophyll a (12-35%) and chlorophyll b (27–28 %) compared to sprouts growing from grey seeds. Seeds treatment with DBD plasma for 3 min had tendency to increase seeds germination, but effect on the germination kinetics was cultivar depended. DBD plasma had no effect on the shoot length and the root weight and length of either brown or grey seeds radish sprouts, but root weight was decreased (12-19% depending on cultivar) by DBD treatment. Seed irradiation with DBD plasma resulted in reduced total content of phenolic compounds (TPC) (by 12%) in sprouts grown from grey seeds in both cultivars, while it did not change TPC in sprouts grown from brown seeds. DBD plasma had no effect on the content of other metabolites in 'Nichinou' seeds sprouts, whereas the same treatment was effective in 'Fujita' sprouts: antioxidant activity in sprouts was increased (by 62% for sprouts from grey and 13% - from brown seeds, compared to control). The content of flavonoids and carotenoids was increased (by 25% and 49%, compared to control), and amount of chlorophyll b was decreased (by 24%), but only in brown seeds sprouts. Thus, seed germination kinetics, early sprout growth and content of different metabolites are dependent on seed color, as well as response to DBD plasma irradiation, but all these differences are also strongly cultivar-depended.

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16. DEVELOPMENT OF HUMAN CALRETICULIN QUANTIFICATION SYSTEM

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Calreticulin (CRT) is an endoplasmic reticulum protein involved in multiple cellular processes. CRT operates as a chaperone and Ca^{2+} transporter in healthy cells to assist in protein folding within the ER. It supports such functions as adhesion, and integrin signaling, and ensures antigen presentation on MHC class I molecules. CRT also has a presence in cell death and immunosurveillance, by appearing on the surface of pre-apoptotic tumor cells to attract phagocytes and initiate immunogenic cell death. There is a debate whether CRT concentration in blood plasma could be a biomarker for neurodegenerative diseases and other processes linked to ageing. There is no widely used standardized CRT quantification assay. Currently, CRT quantification results show variability and are dependent on the CRT quantification assay used. The aim of this research is to develop a well-characterized CRT quantification assay. In this study, mouse monoclonal antibodies (MAbs) to human recombinant CRT (hrCRT) expressed in yeast are used. These MAbs work as pair of capture and detection reagents. The assay is based on sandwich ELISA. CRT concentration is calculated from a measured blood plasma sample optical density by comparing it to standard curve generated by using hrCRT as a standard. The detection range of this test is 0,13-100 ng/mL. In order to characterize the assay, immunoprecipitation of CRT by capture MAb and subsequent detection by detection MAb in Western blot is performed.

17. MAPPING RECOGNITION SITES OF MONOCLONAL ANTIBODIES TARGETING THE SPIKE PROTEIN OF SARS-COV-2

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SARS-CoV-2 virus is responsible for worldwide pandemic that has already cost more than 6 million lives. This virus's spike (S) protein is essential in enabling the virus to enter host cells. Receptor binding domain (RBD) of S protein bind human ACE2 receptor and triggers infusion process after which virus infects the cell[1)]. Therefore, various neutralising and non-neutralising antibodies against S protein are important for research purposes and diagnostic tests. This study aimed to map recognition sites of previously developed ten monoclonal antibodies (MAbs). Six MAbs were generated against recombinant SARS-CoV-2 S protein and four against recombinant RBD. The interaction of the MAbs with six recombinant *Escherichia coli* expressed overlapping fragments of S protein were investigated. Different enzyme-linked immunosorbent assays (ELISA) and western blot (WB) methods were carried out to investigate MAbs recognition sites.
In this study, we identified six out of ten MAbs recognition sites within S protein. MAbs 7E2, 8E11, 14G6 and 3G7 reacted with only one fragment out of six. Thus, MAb 7E2 reactivity with fragment comprising amino acid residues (aa) 171-345 indicated recognition site between 192-317 aa, MAbs 8E11 and 14G6 recognise epitopes within S protein 620-764 aa region. MAb 3G7 recognised fragment located at the end of S protein, indicating its recognisable epitope between aa 790 and 1196. MAb 11A3 recognises two overlapping fragments. Thus, its recognition site is at overlapping aa 620-654 sequence. MAb 5D7 also recognised two overlapping fragments suggesting its recognition site is at 764-790 aa. Four MAbs developed against RBD (11E11, 12B11, 23B5 and 24A12) did not react with any fragments overlapping RBD. However, their epitopes are within RBD between 319 and 541 aa. Our study revealed 6 out of 10 MAbs interaction sites with S protein that span across the whole protein, 7E2 recognised part in the N-terminal domain, in contrast MAbs 3G7 and 5D7 recognised epitopes in S2 domain that is responsible for membrane fusion. MAbs 11A3, 8E11 and 14G6 recognised area around S1/S2 cleavage site.

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18. CHANGES IN COMMON BUCKWHEAT SEEDLING GROWTH AND GRAIN HARVEST INDUSED BY PRE-SOWING SEED TREATMENT WITH COLD PLASMA AND ELECTROMAGNETIC FIELD

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Among the innovative technologies being elaborated for sustainable agriculture, one of the most rapidly developing fields relies on the positive effects of seed treatments with different physical stressors on the agronomic performance of plants. Pre-sowing treatment of common buckwheat (*Fagopyrus esculentum*) seeds with electromagnetic field (EMF) and cold plasma (CP) plasma can induce significant changes in biochemical, physiological processes in growing plants, resulting in increased biomass gain and seed yield. In this study, seeds of common buckwheat (vs. 'Nojai') were treated with low-pressure CP for 2, 5 or 7 min (CP2, CP5 and CP7), vacuum for 5 min (V5), DBD plasma for 2 min (DBD2), and EMF for 2 min (EMF2). Pre-sowing seed treatments had no significant effect on seed germination parameters *in vitro* and emergence in the substrate. After 5 weeks of cultivation in the greenhouse (in plastic boxes filled with the substrate), the photosynthetic efficiency was determined in leaves, seedlings were harvested, their morphometric analysis was performed. The mean values of the efficiency of the second photosystem F_v/F_m ratio was slightly higher only in DBD2 group. The values of performance index PI_{abs} and ET_0/CS_m values in DBD2 and EMF2 groups was 18 – 57%, in comparison to the control. The height of seedlings from EMF2, CP7 and DBD2 groups were 11 - 21%, larger compared to the control, but all treatments had no an effect on above ground weight. DBD2 reduced root length by 26% but increased root mass by 57%. The results of field experiment revealed that pre-sowing seed treatments significantly increased grain yield: the weight of grains per plant in DBD2, CP7 and EMF2 groups was higher by 62, 30, 34%, respectively, compared to the control.

The obtained results confirm that pre-sowing treatment of seeds with physical stressors can increase the growth parameters and harvest yield of common buckwheat, as we showed earlier using different equipment for seed treatment with CP and EMF [1]. It was demonstrated for the first time that DBD plasma treatment is more efficient is this respect, compared to low-pressure CP and EMF treatments.

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19. ISOLATION OF LYSOSOMAL RECOMBINANT PROTEIN TMEM106B

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Neurodegenerative diseases are one of the most widespread in the world, affecting tens of millions of people, medical professionals are predicting numbers will more than triple in the coming 30 years. Recently it was discovered that aggregates of TMEM106B protein are commonly found in some large groups of neurodegenerative diseases, including synucleinopathies, tauopathies, and TPD-43 proteinopathies [1]. TMEM106B is a lysosomal protein highly expressed in neurons and oligodendrocytes. It has 254 amino acid residues and consists of a C, N terminal domains and a single-pass transmembrane domain. Alterations in levels of TMEM106B are linked to neuronal proportions, brain aging, and disorders. Currently, almost all knowledge about the aggregation of TMEM106B comes from post-mortem brain research as purification of the protein is proven hard to do [2]. It is hypothesized that in pathology, unknown proteases cut off the C-terminal domain, and either it aggregates inside lysosomes or it leaves them in an unknown process and forms aggregated structures in the cytosol.
In vitro studies of TMEM106B are limited due to hard-to-replicate purification protocols, low purity levels, and poor yields. Here we aim to purify TMEM106B from genetically modified *E.coli* cells using affinity chromatography and SUMO fusion technology. Fusing SUMO tag to the desired protein is known to promote higher solubility, better folding, and expression. SUMO cleavage results without any additional amino acids at the N-terminus. To that result, the TMEM106B gene was fused at N-termini with ULP1 protease cleavable His-SUMO tag. His-SUMO-TMEM106B gene was inserted into the pET28A expression vector by restriction cloning using BamHI and NdeI restriction endonucleases. Protein was purified using Ni^{2+} ion affinity and size exclusion chromatography methods. Here we aim to purify TMEM106B specific C-terminal fragment (120-254 aa).

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20. TRANSCRIPTOMIC AND PROTEOMIC PROFILING REVEALS THE L-A DSRNA IMPACT ON *SACCHAROMYCES CEREVISIAE* HOST

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Totiviridae L-A virus is a dsRNA virus that persists in *Saccharomyces cerevisiae*. It encodes the primary structural capsid protein Gag as well as the Gag-Pol fusion protein, both of which are required for viral replication and encapsulation. These attributes also allow for the replication of satellite dsRNAs (called M dsRNAs) encoding a toxin and immunity to it (known as killer toxin). The viral capsid pore is thought to be involved in nucleotide absorption and viral mRNA release. Virions stay intracellular and are transmitted to daughter cells during cell division, sporogenesis, and cell fusion.
We report the impact of only L-A virus on gene expression in three related M437 *S. cerevisiae* hosts with varying content of dsRNA viruses using high throughput RNA sequencing data analysis. We present a novel perspective on *Totiviridae* L-A virus-related transcriptional regulation based on multiple bioinformatics analyses. Transcriptional responses to L-A infection were comparable to those caused by stress or food availability. It also investigates the relationship between cell metabolism and L-A virus-imposed demands on the host transcriptome by identifying host proteins that may be connected with intact virions. To get a better understanding of the virus-host relationship, we performed differential proteome analysis on viral particle-enriched fractions of yeast strains containing either the entire killer system (L-A-lus and M-2 virus), M-2 deficient, or virus-free. The major pathways of protein metabolism were found substantially enriched in virus-linked subsets: ribosome biogenesis, folding and proteasomal degradation. These findings substantiate the tight integration of virus with essential pathways of host metabolism, therefore offering background for understanding of LA viral infection persistency.
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21. STRUCTURAL VARIABILITY OF PRION PROTEIN AMYLOID FIBRILS UNDER DIFFERENT AGITATION CONDITIONS

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Amyloidogenic peptides and proteins have a property to convert from their native functional states into fibrillar amyloid aggregates. This property is associated with neurodegenerative disorders, such as Alzheimer's or Parkinson's diseases, as well as prionopathies[1)]. It has been observed that the environment conditions in which amyloid aggregation takes place have an important effect on fibril polymorphism. Moreover, amyloid aggregates have recently been shown to have the property to adopt more than one different conformation under the same environmental conditions[2)]. One example of such environmental conditions, whose effect on amyloid structural variability is not fully understood, is agitation.

In this work, we examined the effect of three different agitation conditions on the aggregation kinetics of mouse prion protein fragment 89-230 (MoPrP) and analyzed the secondary structure of the resulting fibrils.

Protein MoPrP 89-230 samples were incubated under three agitation conditions (200, 400 and 600 RPM) at 37°C, under denaturing conditions (2 M guanidinium hydrochloride, 50 mM sodium phosphate, pH 6.0). The kinetics of aggregation were determined by recording the fluorescence intensity of the amyloidophilic dye thioflavin-T (ThT). The secondary structure of fibrils was determined by analyzing each sample's FTIR spectra.

The results suggest that the intensity of agitation has a minimal influence on primary nuclei formation and that the rate of elongation does to scale with the level of agitation. In all three cases, a diverse collection of secondary structures (at least three structure types) were observed, with the highest variability detected under 200 RPM agitation conditions.

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22. THE MECHANISMS REGULATING BREAST CANCER CELL MIGRATION AND INVASION

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The most crucial challenge in cancer studies are to understand how the cells migrate and regulate in the three-dimensional extracellular matrices during metastasis. (1) In normal conditions, cell migration help to maintain the proper organization of the multicellular organisms, any changes in the mechanism which controls the cell migration will result in extensive issues of cancers and cancer progression from the primary tumour to the secondary tumours. (1,2)
According to recent literature studies, the signalling pathways, membrane trafficking, and cytoskeleton formation are reformed to influence cancer cell migration and metastasis. (3,4)
However, there is a lack of information on how all the processes are coordinated and targeted to specific compartments during metastasis. Thus, adapting Rab40, a small monomeric GTPase that belongs to Ras oncogene mainly functions for the intracellular membrane trafficking, sub-family as an important regulator of the cancer cell migration and metastasis, may contribute to a better understanding of the mechanisms involving Rab40 GTPase and related subfamilies for its relation to the invasion and metastasis. Human breast tumour epithelial cells (MDA-MB-231), together with the knock-out lines of MDA-MB-231 as Rab40a, Rab40b, Rab40c, Rab40abc, Rap2, Rap2 K3R and MDA-MB-231 Cas9 as control cells (expressing the tetracycline-inducible Cas9) were grown. Individual/single-cell migration assay (time-lapse migration analysis) was carried out with cell lines, from which directionality, distance, length and speed were evaluated for each cell line simultaneously. For each single cell migration assay, 15,000 cells were plated on fibronectin-coated 35-mm glass-bottom Petri dishes. Plates were placed in a temperature-controlled chamber with an incubator at 37 °C and images were taken every 20 minutes for 12 h for each cell line, with 4X magnification, in a live-imaging chamber by using the *STEDYCON* microscope and evaluated using *Trackit* software. All the knock-out lines exhibited movement activity, and single-cell migration significantly in a dependent manner for the categories such as the trajectory distance, length, speed, and the persistence of movement called directionality. This method of approach for identifying the potential and functional targets for the breast cancer cell migration, will be useful to identify other families of Rab40 or related to Rab40 such as Rap2, and their role in regulating the cell migration is not fully understood, future studies on Rap2 will be helpful to uncover the function & regulation of Rap2 during the cell migration.

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23. CLONING OF PROTEINS FROM A BACTERIAL FOUR-COMPONENT DEFENSE SYSTEM

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The finding that genes encoding defense systems in prokaryotes typically tend to cluster together in genomic regions called defense islands, led to multiple discoveries of novel, previously unidentified anti-viral systems. Our research is focused on one of many recently discovered defense systems. In a paper published in 2020, this bacterial defense system was able to restrict P1, λ, and T3 dsDNA bacteriophages (Gao et al., 2020). The system encodes a four-gene cluster, containing a KAP family P-loop ATPase, a hypothetical protein of unknown function, a $PreQ_0$ synthase-like protein, and a ssDNA/RNA 3'-5' exonuclease. All of these predictions were made using bioinformatical tools. Based on mutational analysis performed by Gao et al., 2020, $PreQ_0$ synthase-like protein is critically important for anti-viral defense. It is proposed, that $PreQ_0$ might work as a signaling molecule activating ssDNA/RNA 3'-5' exonuclease activity, yet the mechanism of its action remains elusive.
Recently, a new study on phage escapers, that evade bacterial immunity by mutations in phage components, that activate bacterial defense, gave more insight on the system‘s mechanism of action (Stokar-Avihail et al., 2022). An *E.coli* SECphi4 phage was able to form a plaque on bacteria expressing our studied defense system. After isolating, sequencing and comparing the escaper phages genome to a wild-type phage, a mutation was found in a predicted DNA repair exonuclease gene (Stokar-Avihail et al., 2022). Thus, it may be hypothesized that our studied defense system may be triggered by phage replication or its core components.
We aim to characterize the biochemical mechanism of action of this novel defense system. We started by cloning the whole four-gene cassette to pCDF vector under T7- lacO promoter, as well as individual genes with His_6-tag fusions. The whole operon was cloned by Gibson assembly. Cloning of individual system components fusing them with His_6 – affinity tag was performed by restriction enzyme cloning. Recombinant plasmid containing the whole operon under T7-lacO promoter as well as plasmids with individual system components were obtained successfully except ssDNA/RNA 3'-5' exonuclease with C-terminal His_6-tag. Future plans include protein expression and purification.

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24. THE LINK BETWEEN PRO-INFLAMMATORY S100A9 PROTEIN AND TAU PROTEIN

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Neurodegenerative diseases are one of the most common disorders in the world. Unfortunately, despite intensive research, the understanding of the mechanism of these diseases is limited, and almost all existing treatments are symptomatic. Alzheimer's disease is the most common neurodegenerative disease, affecting about 50 million people worldwide. In addition to amyloid plaques composed of amyloid-β peptides, neurofibrillary tangles formed from the protein Tau are a hallmark of this disease and other tauopathies. Moreover, amyloid-β aggregates (and α-synuclein aggregates in Parkinson's disease) have been shown to promote Tau aggregation [1]. It has also been observed that the aggregation of these two peptides involves the pro-inflammatory protein S100A9, which elevated levels in the brain are recorded after various head injuries.
Furthermore, one other tauopathy – Chronic traumatic encephalopathy – registers high levels of Tau aggregates, and the exact reasons for their formation are unknown. This disease is quite prominent in contact sport players who experiences chronic head concussions. There has been some speculation from the scientific community that neuroinflammation could induce Tau pathology; thus, it is feasible that S100A9 as a pro-inflammatory protein could be a culprit behind it or at least in part responsible. However, it is strange that there is not much information available or studies performed to confirm or rule out the potential of the S100A9 protein or its aggregates to participate directly in Tau aggregation [2]. Therefore, we examined the ability of the S100A9 protein and its aggregates to promote Tau aggregation. We observed that Tau aggregation is dependent on S100A9 aggregate formation as S100A9 monomers alone do not induce Tau aggregation, while S100A9 aggregates induce notable fluorescence changes in the reaction mixture with Tau protein. Various conditions for S100A9 protein aggregation were examined in the study.

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25. STRUCTURAL REASERCH OF CRISPR-CAS ANCILLARY PROTEIN

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Type III CRISPR-Cas system provides prokaryotes with adaptive defence against viruses. It was noted that these systems employ specialized cell signalling pathways to combat infections. When CRISPR-Cas effector complex recognizes foreign RNA, it starts to synthesize cyclic oligoadenylates from ATP1. These molecules activate diverse ancillary proteins which consist of signal recognition domain and effector domain: RNase, DNase, ATPase, protease or others2. Functions and structures of several effector domains have been elucidated: for example, the RNase activity of HEPN domains3,4 and the DNase activity of restriction endonuclease like effectors5,6. However, many other effector domains have not been studied. Recent sequence analysis revealed that a group of such effectors are similar to translation inhibiting toxins from bacterial toxin-antitoxin systems2. We present crystallization results for toxin related to CRISPR-Cas system and discuss possible strategies for upcoming studies.

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26. ACTIVATION OF MICROGLIAL NLRP3 INFLAMMASOME BY IMMUNE COMPLEXES OF VIRUS-LIKE PARTICLES

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The inflammasome is an important component of innate immunity. The best-described inflammasome is NLRP3, which contains three major components – nucleotide-binding and oligomerization domain-like receptor, adapter protein apoptosis-associated speck-like protein (ASC) and caspase-1[1)]. NLRP3 inflammasome activation results in release of inflammatory cytokines, like IL-1β, and inflammatory cell death – pyroptosis[2)]. In our previous research we showed that viral proteins triggered NLRP3 inflammasome activation depending on their structure[3)]. The aim of this study was to extend the latter research and determine whether immune complexes of oligomeric proteins could change the NLRP3 inflammasome activation in macrophages.

Primary mouse microglia were selected as cell culture model. Cells were treated with spherical virus-like particles (VLPs) of these human polyomaviruses (PyV): KIPyV, WUPyV, and their immune complexes. MCC950 was used to inhibit NLRP3 inflammasome activation. NLRP3 activation was studied by evaluating cell viability, IL-1β and TNF-α cytokine release and the formation of ASC specks.

It was found that spherical PyV-derived VLPs and their immune complexes induced cell death, IL-1β secretion and ASC speck formation in microglia indicating NLRP3 inflammasome activation. In addition, immune complexes mediated a significantly higher cellular response compared to VLPs alone. To conclude, our results demonstrate that immune complexes can enhance inflammasome activation.

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27. THE EFFECT OF LONG-TERM SUPPLEMENTATION WITH ALUMINUM OR SELENIUM IONS ON ANTIOXIDANT ENZYMES ACTIVITIES

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In our previous studies, we evaluated the effect of acute exposure of Al ions on oxidative stress and the capacity of the antioxidant system in mouse organs by using the Al intoxication model that involved the injection of $AlCl_3$ solution into the abdominal cavity of the mouse. However, the obtained results encouraged us to select a different route of administration of Al: the oral administration. This is a natural route of entry of Al into the body. In addition, such administration does not cause inflammation at the site of the injection. Several studies performed with experimental animals have demonstrated changes in the cognitive functions and morphological peculiarities of the CNS following the consumption of water with elevated concentrations of Al. Even through the absorption of Al though the gastrointestinal system is very poor, a long-term negative effect of Al cannot be ruled out completely, even if the concentrations of Al that enter the body with potable water are lower.
The aim of this study was investigated the effects of Al or Se ions on the “primary” antioxidant defense system enzymes in mice brain and liver after 8-week exposure of drinking water supplemented with $AlCl_3$ (50 or 100 mg Al/L) or Na_2SeO_3 (0.2 or 0.4 mg Se/L).
The experiments done on BALB/c laboratory mice. The antioxidant enzymes activities in mice brain and liver homogenates determined spectrophotometrically. Results expressed as the mean ± SEM.
The results shown that higher dose of Se increased the activities of superoxide dismutase and catalase in both mouse organs. Exposure to lower dose of Se resulted in an increase of catalase activity in mouse brain but did not show any statistically significant changes of superoxide dismutase activity in both organs. Meanwhile, the administration of both doses of Al caused no changes in activities of these enzymes in mouse brain and liver. The greatest sensitivity to the effect of Al or Se was exhibited by glutathione reductase. Exposure to both doses of Al or Se resulted in statistically significant increase of glutathione reductase activity in both brain and liver.
It was concluded that long-term exposure of se ions caused statistically significant increase of superoxide dismutase, catalase, and glutathione reductase activities in mouse brain and/or liver, however, these changes were dependent on the used dose. The exposure to both al doses caused statistically significant increase only activity of glutathione reductase in both organs.

28. STUDY OF POTENTIAL NEW BACTERIAL DEFENSE SYSTEMS TARGETING NUCLEIC ACIDS

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Bacteria, the most abundant form of life on Earth, face many opponents during its life. The main opponents of bacteria include bacteriophages and foreign plasmids. Bacteria have numerous different molecular mechanisms of defense against these invaders. The immunity can be adaptive like CRISPR-Cas systems, or DNA modification based, like Pgl systems, defense system may also work by inducing programmed cell death.

A variety of defense systems in the bacterial genome are found together in clusters called defense islands. Bioinformatic analysis of one of these prokaryotic genome regions lead to a potential new defense system being found. The mechanism was discovered by comparing already known sequences associated with bacterial defense to a sequence of interest. We are working with three types of this new potential bacterial defense system. The main domains of this system potentially having a significant activity during the invasion of a bacterial cell are McrB and McrC. McrB is believed to be important for DNA translocation, McrC on the other hand has a nuclease activity domain [1]. Two types of this newly found potential system also have TerY-P triad which occurs in other known defense systems[2].

The main goal of this study is to determine the effectiveness, or lack thereof, of this potential defense system against bacteriophages and plasmids. To test our hypothesis, we performed experiments on transformed *E. coli* cells with different types of a potential defense system. To examine anti-viral activity small drop plaque assays on double-layer plates have been evaluated, although, no significant activity has been shown with various phages. Next, bacteria were challenged with different plasmids, to determine if the potential defense system blocks foreign plasmid transformation into the cell. As shown by previous studies some defense systems target invading plasmids, such as the pAgo system against multi-copy plasmids[3] or DdmABC and DdmDE systems[4]. Initial data shows that there possibly is an effect against some of the invading plasmids. This has been shown by performing plasmid interference assays. The results are promising but unclear – further experiments will be performed to assess our hypothesis.

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29. INSIGHT INTO THE HETEROGENEITY OF PROSTATE CANCER THROUGH MULTIGENE TESTING

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In Lithuania, approximately 2,000 men are diagnosed with prostate cancer (PCa) yearly. Prostate-specific antigen is one of the most common tools used for diagnostic purposes in PCa, however its specificity remains extremely low. Since, it is assumed that about 60% of individual variation in PCa risk can be attributed to genetic components, we believe that genetic testing in conjunction with other screening tools can improve diagnostic efficiency and risk assessment of PCa.
35 genes were selected for multigene testing based on their significance for disease development and interface with an increased risk of developing the disease. NGS technology was applied for genomic profile analysis of 61 PCa cases from National Cancer Institute. All identified mutations were validated with Integrative Genomics Viewer, ClinVar, COSMIC and VarSome databases and classified as pathological, non-pathological, and risk factors.
Pathological mutations, such as c.1813delA and c.470T>C, were detected respectively in *BRCA2* and *CHEK2* genes. At least one PCa risk-related SNP was observed for each patient. Furthermore, we identified that patients with higher grade cancer had significantly more risk associated SNPs and pathogenic mutations compared with lower grade patients ($P>0.04$). We also determined association between lower apparent diffusion coefficient (ADC) value, which is a important imaging biomarker that is strongly associated with higher T stages, and bigger alteration count ($P=0.01$).
In conclusion, this genetic profiling revealed significantly higher prevalence of potentially pathogenic variants and SNPs related to a higher risk of the disease in patients with higher grade cancer and lower ADC. Thus, the multigene diagnostic system in conjunction with clinical data has the potential to allow proper prediction of disease risk and progression and reduce overdiagnosis of clinically insignificant cancer.

30. DYSREGULATED URINARY MIRNAS AND THEIR ASSOCIATION WITH TREATMENT RESISTANCE IN TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype. It lacks expression of progesterone, estrogen, and human epidermal growth factor 2 receptors. Due to the lack of therapeutic targets, TNBC has limited treatment options [1]. In recent years it has been shown that many different microRNAs (miRNAs) are dysregulated in cancer cells and human body fluids. Moreover, it has been discovered that ~30% of nonurinary molecules are passed through the kidneys. It suggests that urine could be a promising resource for non-invasive biomarker research for nonurological cancers such as TNBC [2]. Furthermore, an individualized comprehensive treatment strategy based on non-invasive biomarkers such as urinary miRNAs could be helpful in treatment prediction for prolonged progression-free survival of TNBC patients. This research aimed to identify dysregulated urinary miRNAs associated with chemoresistance in TNBC, which could help in treatment prediction.

To evaluate miRNAs expression differences in serial urine samples, we used RT-qPCR. In more than 50 serial TNBC patients' samples, urinary levels of 3 miRNAs were examined (miR-200a-3p, -210-3p, -125a-5p). The samples were collected before and 6 months after neoadjuvant chemotherapy treatment. MiR-200a was upregulated up to 1.2 times before treatment. Besides, miR-200a-3p, -210-3p were upregulated up to 3 times in the progressive disease patients' group compared to the stable disease patients' group. This suggests that our analyzed urinary miRNAs could be promising candidate biomarkers for response to chemotherapy monitoring in TNBC.

Overall, miR-200a-3p, -210-3p, -125a-5p could be involved in resistance to chemotherapy mechanisms. Identification of miRNAs level differences in TNBC patients' urine specimens has the potential to be used as a non-invasive method that would help predict the efficacy of treatment.

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31. NANOPORE SEQUENCING-BASED ANALYSIS OF MODIFIED DNA

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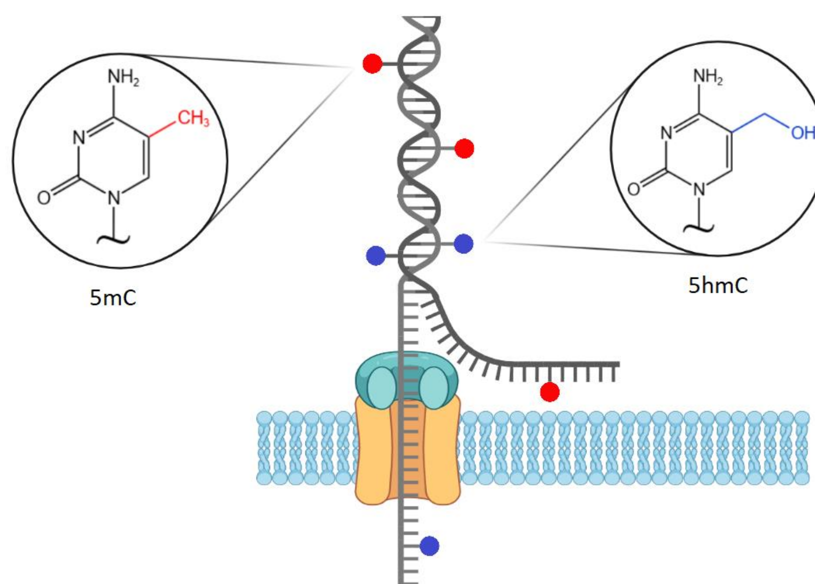
Every cell of a multicellular organism carries the same genome. Amazingly though, epigenetic regulation of gene expression drives cell differentiation towards various cell types, with dedicated functions and features. One of the fundamental epigenetic mechanisms is DNA modification. In the mammalian genome, cytosines in CpG dinucleotides are often methylated to give 5-methylcytosine (5mC) which is usually associated with silenced chromatin. This epigenetic mark is brought about by specific enzymes DNA methyltransferases. Further, through the action of oxygenase TET proteins, 5mC can be converted to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) which are further recognized by regulatory proteins[1]. Some lower eukaryotes use 6-methyladenine (6mA) as an epigenetic mark too. Moreover, the versatility of modified DNA bases is even wider in prokaryotes and viruses.

DNA modification repertoire is not limited to the natural entities though. Biotechnological approach has developed an assay called mTAG (methyltransferase-directed Transfer of Activated Groups) where engineered DNA methyltransferases can site-specifically introduce extended alkyl groups. The approach has been used for site-specific DNA labelling with a multitude of applications, ranging from optical genotyping to whole genome methylation profiling[2].

The rapidly advancing Nanopore Sequencing technology has a unique perspective of ability to detect modified bases directly in the native DNA sequence at a single-base resolution. We are taking advantage of using a simple MinIon device from Oxford Nanopore Technologies to detect modified bases, both natural and biotechnologically engineered, in model DNA sequences. Further, we are open for collaboration in analysis of any modified DNA relevant to your scientific interests.



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32. HIGH CONTENT ANALYSIS OF SEA BUCKTHORN AND CHERRIES-ASSOCIATED MYCOBIOTA

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Sea buckthorn (*Hippophae rhamnoides L.*), sour cherries (*Prunus cerasus L.*) and sweet cherries (*P. avium L.*) are economically important fruits with high potential in the food industry and medicine. Sea buckthorns and cherries are highly colonized by various bacterial and fungal microorganisms with potential pathogenic and beneficial features. The plant-associated yeast community year-to-year is characterized by the appearance of many new patterns, related to plant species, ripening stage, environmental changes, climatic conditions and other factors. In this study, we analyzed and compared fungal microorganism communities associated with the carposphere of sea buckthorns, sour and sweet cherries that were freshly harvested from private plantations. Following DNA isolation, a DNA fragment of the ITS2 rRNA gene region of each sample was individually amplified and subjected to high-throughput Next Generation Sequencing (NGS).
Mycobiota of sour and sweet cherries was more diverse than sea buckthorns at the genus level. Bioinformatic data showed significant similarities between dominant yeast on sea buckthorn and cherries – *Aureobasidium* and *Metschnikowia* prevailed on all berries. Investigation of microbial ASVs diversity revealed plant-dependent fungal microorganism assemblages. Among the microorganisms inhabiting tested berries, potentially advantageous (*Hanseniaspora*, *Metschnikowia,* etc.), possessing biocontrol features, or dangerous fungal microorganisms (*Rhodotorula*, *Vishniacosyma*, *Filobasidium*, etc.) were detected. Heat maps illustrate the distribution of the most abundant fungal ASVs and reveal community composition. Based on the distribution pattern of dominating ASVs, similarities of fungal communities between sour and sweet cherries were detected. Our study providing valuable data on the differences in mycobiota structure of tested berries are important for the development of the strategies for plant cultivation and disease management.
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33. RNA SEQUENCING OF TWO BREAST CANCER CELL LINES IN COMPARISON TO HEALTHY EPITHELIAL BREAST CELLS

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We studied genes, that are differentially expressed between malignant and normal breast tissue, to find weak spots for anti-cancer therapy development. RNA sequencing of three cell lines was performed: MCF-7 (epithelial breast cancer cell line), BCC (primary breast tumour cell line) and MCF-10A (epithelial breast cell line). Raw data was aligned and normalized read frequencies of the genes were calculated using a customized Python script with HTSeq and other packages. Given values were corrected for multiple testing using the Benjamini-Hochberg method.
A total of 3147 genes were found to be upregulated and 2440 were downregulated in malignant cell lines, in comparison to MCF-10A. Of them 274 upregulated and 563 downregulated genes were found in both MCF-7 and BCC, meaning they could be relevant to breast cancer. Some of the most significantly overexpressed genes in both cancer cell lines were Serine Protease 23 (PRSS23) and Tripartite Motif Containing 37 (TRIM37) genes, that are highly associated with breast cancer in literature [1,2]. Many of the shared largely upregulated genes in breast cancer cell lines were also linked to neurodegenerative diseases in literature[3]: PADI, APP, PSAP, SGK1, CLU, APLP2, MT-ND5. This means further investigation of these diseases can answer several questions at once or could be used to find treatment in existing medication for both by using them for other indications. Other genes, that were highly upregulated in one or the other of the two breast cancer cell lines include: PLK2, CYR61, IGFBP4, EEF1A2 (in BCC), TPM1 (both in BCC and MCF-7), TFF1, KRT18, CSDE1, XBP1, PSMD6, EIF4B (in MCF-7).
The genes mentioned in this study hint important processes for breast cancer to thrive. Further research of their functions may provide hope for future chemotherapy.

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34. MONOCLONAL ANTIBODIES AGAINST HOUSE DUST MITE ALLERGEN DER P 21 AND THEIR APPLICATION FOR THE ANALYSIS OF ALLERGEN EXTRACTS

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The house dust mite (HDM) is one of the most important and widely spread allergen sources causing allergy and its related complications. Recombinant allergens and HDM extracts are being used in allergy diagnostics and immunotherapy. Since allergen extracts from different manufacturers lack proper standardization regarding to their composition, monoclonal antibodies (MAbs) against specific allergen components can be used for their identification and quantification in allergen extracts.
This study aimed to develop recombinant allergen Der p 21 of *Dermatophagoides pteronyssinus* and generate MAbs against this allergen. Der p 21 allergen fused with maltose-binding protein (MBP) was expressed in *E. coli* and purified using affinity chromatography of MBP-passenger proteins. The fused protein contained TEV protease cleavage site between the allergen and MBP and the hexahistidine sequence. Hydrolysis reaction was optimized to detach MBP and recombinant Der p 21 was purified using affinity Ni-NTA chromatography.
To investigate the antigenic properties of recombinant Der p 21, its reactivity with blood serum specimens of patients with diagnosed HDM allergy was analyzed. Recombinant Der p 21 was recognized by HDM-specific IgE thus confirming its antigenic similarity with native allergen. Therefore, recombinant purified Der p 21 was a suitable antigen for MAb generation. Five hybridoma cell lines producing high affinity MAbs of IgG isotype were generated using hybridoma technology. Highly specific sandwich ELISA for the quantification of Der p 21 was developed and optimized. Three HDM allergen extracts from different manufacturers were analysed using the newly developed sandwich ELISA and Western blotting. The component Der p 21 was detected in only two of the extracts revealing significant differences of allergen composition. These data demonstrate the importance of allergen-specific MAbs as a tool for the characterization of allergen extracts and the need of an appropriate standardization of extracts before their use for allergy diagnostics or immunotherapy.

35. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST OXA-48 AND OXA-134 β-LACTAMASES

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Antimicrobial resistance (AMR) is the largest global health threat in the 21st century and it requires urgent measures. Major reasons behind the emergence of AMR - misusing and overusing different antibacterial agents in the healthcare settings [1]. One of the steps in limiting the spread of AMR is accurate diagnostics of the disease and prescription of effective antibiotics. Currently applied diagnostic methods include phenotyping, biochemical and genotyping methods which tend to be expensive and protractive. Immunodetection, on the other hand, is rapid, specific, and sensitive method for identifying resistance factors. However, development of immunodiagnostic tests is limited by lack of highly specific monoclonal antibodies (MAbs).
The aim of this study was to generate and characterize MAbs against two antibiotic resistance factors – OXA β-lactamases OXA-48 and OXA-134, which are widespread in pathogenic *Enterobacteriaceae* and confer resistance to carbapenems [2]. Using hybridoma technology one specific anti-OXA-48 MAb and three specific anti-OXA-134 MAbs secreting cell lines were created. All four generated MAbs were shown to have high affinity towards respective antigens and recognize their linear epitopes. One anti-OXA-134 monoclonal antibody pair was shown to have potential to be applied in antibiotic resistance factors detection tool.

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36. DEVELOPMENT, CHARACTERIZATION AND APPLICATION OF MONOCLONAL ANTIBODIES AGAINST SARS-COV-2 NUCLEOCAPSID PROTEIN

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Effective diagnostic systems detecting new cases of COVID-19 are essential for stopping the spread of human coronavirus SARS-CoV-2. Due to the early and abundant synthesis of SARS-CoV-2 nucleocapsid during infection, SARS-CoV-2 N protein is considered a potential serological marker for application in diagnostic systems. This research aims to develop monoclonal antibodies (MAbs) specific to SARS-CoV-2 virus N protein and adapt them to form a sandwich enzyme-linked immunosorbent assay (ELISA) system. Due to the close homology of the N-terminal part of nucleocapsid to other coronaviruses, to avoid non-specific antibody interactions with them a truncated version of the N protein ($N_{121-419}$) was used to immunize experimental animals. Evaluation of mice's humoral response revealed that after the immunization plasma cells are secreting antibodies specific to SARS-CoV-2 $N_{121-419}$ and SARS-CoV-2 N antigens. Using hybridoma technology, mice spleen cells were used to generate cell lines secreting MAbs specific both to SARS-CoV-2 N and $N_{121-419}$. The interactions and identification of favorable combinations between MAbs of the generated collection were evaluated by the competitive ELISA method. Based on the obtained results, thirty pairs of antibodies were selected and tested by the sandwich ELISA method. Eleven new MAbs combinations capable of attaching and visualizing SARS-CoV-2 N antigen in solution were confirmed. Additionally, for previously developed coating 4G6 MAb, which is specific to full-length SARS-CoV-2 N, two alternatives anti-SARS-CoV-2 $N_{121-419}$ MAb-HRP compatible conjugates were also detected. Considering these results, the usage of truncated SARS-CoV-2 nucleocapsid protein is equally effective in the development of specific MAbs with the benefit of avoiding interaction with homologous proteins of other coronaviruses. This research was carried out as part of the LMT research group project "Development of diagnostic antibodies against SARS-CoV-2" no. 13.1.1-LMT-K-718-05-0031. The project is financed by the European Regional Development Fund as the European Union's response to the COVID-19 pandemic measure no. 13.1.1-LMT-K-718 "Targeted research in the field of skillful specialization".

ORAL PRESENTATION ABSTRACTS

PROTEIN STRUCTURE MODELING USING ALPHAFOLD: WHAT WORKS AND WHAT DOES NOT

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AlphaFold is a machine learning-based protein structure prediction method that can generate structure models with an accuracy comparable to experimental methods[1)]. Knowing that the ability to predict protein structures has been desired for a long time, it is not surprising that the release of AlphaFold in 2021 was met with huge hype. In addition to the modeling of monomeric protein structures, AlphaFold was immediately applied for various tasks[2)]: obtaining models for molecular replacement in crystallography and for cryo-electron microscopy, modeling of multimeric protein complexes and protein-peptide interactions, estimating protein oligomeric states and predicting possible interactions, etc. More recently, monomeric structures of almost all known proteins were predicted[3)] and deposited in AlphaFold database[4)], and the resulting models are used in efforts to characterize protein functions. As a result, it is important for everyone to know what AlphaFold and other modern protein structure modeling methods can do, and what is not yet possible.

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STEAM EDUCATION: SITUATION IN LITHUANIA

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The successful implementation of molecular life sciences study programs in universities is highly impacted by the quality and quantity of the product of the previous stages of the education system - school graduates. Most countries report critical decrease in the number of students entering university science, maths and technology programmes. It was recognized that such situation is predetermined by the traditional subject-based education, which is based on the instructive approach and mostly theoretical teaching. In contrast, the STEAM curriculum aims to increase the attractiveness of natural and technological sciences, to stimulate students' interest in these sciences, and increase their motivation to choose STEAM-related studies. However, the experience of different countries show it is much easier to declare about intentions to introduce STEAM education than to make it function.

In terms of the content, the English acronym STEAM includes Science, Technology, Engineering, Arts/All other sciences and Mathematics. Alas, it is not enough to list the meaning of the letters that make up the abbreviation. The term is used to refer to educational policy and curriculum selection in schools. STEAM innovations are not in the teaching content but in the teaching process.

It is important to understand that the teaching process is STEAM training is used to supplement, apply and systematize the knowledge gained during subject learning, which differs from the traditional one in many aspects: (1) STEAM subjects are integrated into integrated practical activities. This aims to form a comprehensive (holistic) knowledge of phenomena and the ability to solve real-life problems; (2) Traditional teaching methods based on lessons and reading textbooks are replaced by active learning based on research, problem-based learning, team and authentic project activities. Experience on attractive research helps to understand scientific concepts and the principle of scientific research, and results in positive attitude towards science; (3) STEAM education is aimed at developing students' initiative and independent learning abilities. The role of the teacher and the teaching methodology are changing - the teacher is not a provider of theoretical knowledge, but a facilitator of active learning; (4) The importance of applying knowledge and skills to real life is emphasized, teaching topics are connected to the everyday life of students (contextuality); (5) STEAM education takes place everywhere - both in school and outside. Both formal and informal forms of education are applied; the school community, families, school partner universities and business enterprises are involved; (6) Scientific literacy, rational and critical thinking are developed; learners are prepared for integrated careers, real work activities that require problem-solving skills, integrated application of knowledge of various subjects and team work. STEAM education does not replace traditional subject teaching, but complements it and is combined with it. In the context of the ultimate need to increase the number of students entering programmes of natural science in universities, the current situation with STEAM education in Lithuania will be presented and discussed.

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