TAL TECH

FEBS3+ Conference of Estonian, Latvian and Lithuanian Biochemical Societies

ABSTRACT BOOK

Tallinn, Estonia
15–17 June 2022

FEBS3+ Conference of Estonian, Latvian and Lithuanian Biochemical Societies

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The conference was organized by Estonian Biochemical Society in collaboration with Lithuanian Biochemical Society, Latvian Biochemical Society and support from FEBS.

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Dr Tiit Lukk, President of the Estonian Biochemical Society, Tallinn University of Technology

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CONFERENCE BACKGROUND

The FEBS3+ Meetings programme was established by FEBS to support scientific meetings, equivalent to an annual national scientific meeting of a Consituent Society, but organised through the collaboration of at least three FEBS Constituent Societies. The 2022 FEBS3+ conference is taking place in Tallinn and is organized by Estonian Biochemical Society together with Latvian and Lithuanian Biochemical societies and is further supported by Tallinn University of Technology, Estonian Academy of Sciences and sponsors from industry. The 2022 Tallinn FEBS3+ conference is the second in series of joint meetings of Baltic Biochemical Societies as supported by FEBS.

CONFERENCE PROGRAMME

Wednesday, June 15

9:00 REGISTRATION AND COFFEE

10:00 Opening ceremony – **Tiit Lukk** (Estonian Biochemical Society)

10:05 **Prof. Tiit Land** (Tallinn University of Technology) – Welcome

10:10 **Prof. Jerka Dumic** (University of Zagreb, Faculty of Pharmacy and Biochemistry, Croatia; Federation of European Biochemical Societies) „FEBS –advancing molecular life sciences"

Plenary Session – Session chair: Kaspars Tars

10:30 **Prof. Marc Baumann** (University of Helsinki, Finland; Finnish BioBio) „Clinical proteomics *Quo Vadis*? Fine-tuning clinical proteomics for the future"

10:50 **Prof. Daumantas Matulis** (Vilnius University, Lithuania; LBS) „Fluorescent probes for CAIX expressed in live cancer cells"

11:10 **Prof. Kristaps Jaudzems** (Latvian Institute of Organic Synthesis) „Structural analysis of an antigen chemically coupled on virus-like particles"

11:30 FEBS National Lecture presented by the Latvian Biochemical Society: **Prof. Helgi Schiöth** (Uppsala University, Sweden) „Human drug targets: trends in drug discovery"

12:15 LUNCH

Session 2 – Session chair: Asko Uri

13:10 **Dr. Kadri Ligi** (Solis BioDyne OÜ) „Birth of a protein: from development to production"

13:30 **Dr. Mart Ustav Jr** (Icosagen Cell Factory OÜ) „Inhalation based delivery of broadly neutralizing SARS-CoV-2 antibodies"

13:50 Prof. Rimantas Daugelavicius (Vytautas Magnus University) „Potentiometry for studies of membranes and energetics in microorganisms“

14:10 Dr. Vytautas Petrauskas (Vilnius University) „A comprehensive tool for protein-ligand binding constant determination by thermal shift assay“

14:30 Tõnis Laasfeld (University of Tartu) „Live cell fluorescence-based ligand binding assays powered by automated microscopy and machine learning image analysis“

14:45 COFFEE BREAK / POSTERS

Session 3 – Session chair: Vytautas Petrauskas

15:15 Dr. Kaisa Marjamaa (VTT) „Enzymatic fibre modifications: from fundamentals to applications“

15:35 Prof. Priit Väljamäe (University of Tartu) „H_2O_2 cosubstrate is a double-edged sword for lytic polysaccharide monooxygenases (LPMOs)“

15:55 Prof. Angela Ivask (University of Tartu) „Antimicrobial materials and surface coatings: an effective solution or a matter of concern“

16:15 Prof. Kaspar Valgepea (University of Tartu) „Unraveling acetogen gas fermentation using quantitative systems biology“

16:35 Prof. Petri-Jaan Lahtvee (Tallinn University of Technology) „Developing locally applicable biotechnology value chains“

17:00 POSTER SESSION

20:00 GALA DINNER

Thursday, June 16

Session 4 – Session chair: Ly Villo

9:00 **Prof. Mart Saarma** (University of Helsinki) „ER stress regulation CDNF and MANF proteins protect cells by novel mechanism"

9:20 **Dr. Aiste Jekabsone** (Lithuanian University of Health Sciences) „Extracellular vesicles as inflammatory mediators between airway and brain"

9:40 **Prof. Andres Salumets** (University of Tartu) „How knowledge of biochemistry can help clinical medicine: an example of reproductive medicine"

10:00 **Prof. Aija Linē** (Latvian Biomedical Research and Study Center) „Functional roles of exercise-induced extracellular vesicles in the prevention of cancer"

10:20 COFFEE BREAK / POSTERS

Session 4 continued – Session chair: Daumantas Matulis

10:50 **Dr. Zane Kalnina** (Latvian Biomedical Research and Study Centre) „Development of an *in vivo* model system for carbonic anhydrase 9 targeting in breast cancer"

11:10 **Dr. Davids Fridmanis** (Latvian Biomedical Research and Study Cenrte) „Detection of SARS-CoV-2 RNA in wastewater: importance of viral lineage and population size assessment"

11:30 **Kristina Mašalaite** (Vilnius University) „NLRP3 inflammasome activation by immune complexes of virus-like particles in microglia"

11:45 **Juta Rainytė** (Vilnius University) „Immunological comparison of recombinant shrimp allergen Pen m 4, produced in *Pichia pastoris* and *Escherichia coli*"

12:15 LUNCH

Parallel session 5a – Session chair: Maija Dambrova

13:00 Dr. Vaida Šeputienė (Thermo Fisher Scientific Baltics UAB) „A new medicine in your prescription – mRNA"

13:20 Dr. Baiba Svalbe (Latvian Institute of Organic Synthesis) „Stealth brush polymers avoid rapid clearance and display effective accumulation in TNBC tumours"

13:40 Dr. Valeryia Mikalayeva (Lithuanian University of Health Sciences) „Alternative energy: The role of branched-chained amino acids in breast cancer cells"

14:00 Danilo Mladenović (Tallinn University / Hansabiomed Life Sciences) „Acidification of blood plasma facilitates the isolation and analysis of extracellular vesicles"

Parallel session 5b – Session chair: Ago Rinken

13:00 Dr. Emilio Parisini (Latvian Institute of Organic Synthesis) „Drugging the undruggable: towards the development of selective modulators of cadherin-mediated cell-cell adhesioon"

13:20 Dr. Tarvi Teder (Karolinska Institute) „Structural, functional and regulatory aspects of leukotriene biosynthetic enzymes"

13:40 Dr. Edijs Vavers (Latvian Institute of Organic Synthesis) „Interaction between SIGMA-1 and GABA-B receptors: evidence from SIGMA-1 receptor knockout mice"

14:00 Maris-Johanna Tahk (University of Tartu) „Methods for measuring ligand binding properties to Neuropeptide Y Y1 receptors with novel high-affinity fluorescent ligands"

14:30 COFFEE BREAK / POSTERS

Session 6a – Session chair: Tiit Lukk

15:00 Prof. Kaspars Tars (Biomedical Research and Study Center, Riga, Latvia; LaBS) „Structure and applications of ssRNA bacteriophage AP205"

15:20 Dr. Gints Kalnins (Latvian Biomedical Research and Study Center) „Variety of GRM2 type bacterial microcompartment particles demonstrated by cryo-EM"

15:40 Dr. Karin Ernits (Lund University) „Small alarmone (p)ppGpp hydrolase SpoT counteracts the synthetase activity of RelA"

16:00 Dr. Teodors Pantelejevs (Latvian Institute of Organic Synthesis) „A conserved mechanism of interferon signalling antagonism in poxviruses and paramyxoviruses"

16:20 Dr. Priit Eek (Pennsylvania State University) „Structural studies of chromatin proteins: the impact of cryo-EM"

17:00 POSTER SESSION

Session 6b – Session chair: Reet Kurg

15:00 Dr. Egils Stalidzans (University of Latvia) „Interactions of kinetic and stoichiometric models in the estimation of flux distribution in a metabolic Network"

15:20 Paola Monteiro de Oliveira (Tallinn University of Technology) „Growth characterization and substrate consumption patterns of non-conventional yeasts in C5-enriched hydrolysates from birch"

15:35 Alina Rekena (Tallinn University of Technology) „Metabolic network analysis of *Rhodotorula toruloides* using stoichiometric models and proteomics analysis"

15:50 Henrique Sepúlveda Del Rio Hamacek (Tallinn University of Technology) „Development of 3D printed engineered living materials for biochemicals production"

16:05 Dalia Smalakyte (Vilnius University) „Self-regulation of the type III Crispr-Cas signaling pathway"

16:20 Hegne Pupart (Tallinn University of Technology) „Insights into bacterial dye-decolorizing peroxidases and their lignin remodeling activity"

Friday, June 17

Session 7 – Session chair: Tiit Land

9:00 **Prof. Vilmante Borutaite** (Lithuanian University of Health Sciences) „Toxic when being outside: how extracellular tau proteins kill neurons"

9:20 **Dr. Vytautas Smirnovas** (Vilnius University) „The role of environment in amyloid aggregation"

9:40 **Prof. Peep Palumaa** (Tallinn University of Technology) „Copper metabolism in Alzheimer's disease"

10:00 **Dr. Alons Lends** (Latvian Institute of Organic Synthesis) „Functional and pathological amyloids seen by solid-state NMR spectroscopyd-state NMR"

10:20 **Dr. Sebastian Wärmländer** (Stockholm University) „Cell-penetrating peptides with unexpected anti-amyloid properties"

10:40 COFFEE BREAK / POSTERS

Session 8 – Session chair: Kaspars Tars

11:30 **Dr. Rasa Zukiene** (Vytautas Magnus University) „Modulation of Stevia secondary metabolism with cold plasma"

11:50 **Dr. Janis Liepins** (University of Latvia) „Purine auxotrophic starvation evokes quiescence like phenotype in the budding yeast"

12:10 **Dr. Edgars Liepins** (Latvian Institute of Organic synthesis) „Long-chain acylcarnitines: from mitochondrial metabolism to clinical applications"

12:30 **Tanel Sõrmus** (University of Tartu) „Covalent bisubstrate inhibitors of protein kinases"

12:45 **Robert Risti** (Tallinn University of Technology) „Albumin affects the stability, oligomerization and ligand interactions of lipoprotein lipase"

13:00 Best poster award ceremony, concluding remarks

13:15 LUNCH

POSTERS

The number before the poster (1-1-16) represents:
1 (number of the poster) – 1 (number of the stand at the poster area) – 16 (poster presented on June 16). 15/17 or 16/17 the poster will be presented on both days (all student posters).

1-1-16 Dr. Nikhil Agrawal (Latvian Institute of Organic Synthesis, Latvia) „Investigating the early stages of misfolding of amyloid-forming peptide PAP248-286 using molecular dynamics simulations"

2-2-15 Dr. Dominykas Aleknavičius (Nature Research Centre, Lithuania) „Metagenomic analysis of bacterial communities of the edible insect the house cricket"

3-3-16 Dr. Egle Balciunaite (National Cancer Center, Lithuania) „miRNAs as potential biomarkers for lung cancer"

4- 4-15 Dr. Ina Balke (Latvian Biomedical Research and Study Centre, Latvia) „Ryegrass mottle virus protease structure and proteolytic properties"

5-5-16 Prof. Rasa Baniene (Lithuanian University of Health Sciences, Lithuania) „The effect of hypoxia/reoxygenation on renal cells mitochondrial function"

6-2-16/17 Elina Berntsson (Tallinn University of Technology, Estonia) „Mercury ion binding to Apolipoprotein E variants ApoE2, ApoE3, and ApoE4: similar binding affinities but different structure induction effects"

7-1-15/17 Shapla Bhattacharya (Latvian Institute of Organic Synthesis, Latvia) „Enzyme Engineering of fructosyl peptide oxidase to widen its active site access tunnel and improve its thermal stability"

8- 7-16 Jhon Alexander Rodriguez Buitrago (Latvian Institute of Organic Synthesis, Latvia) „Molecular and structural bases of the Plasmodium falciparum threonyl-tRNA synthetase inhibition"

9-9-16 Dr. Rossella Castagna (Latvian Institute of Organic Synthesis, Latvia) „Photoswitchable ligands as tool to modulate biological activity"

10-3-15/17 Enrika Celitan (Life Sciences Center, Lithuania) „Synthesis and assembly of *Saccharomyces cerevisiae* L-BC virus-like particles"

11-11-16 Ugne Drazdauskiene (Thermo Fisher Scientific Baltics UAB, Lithuania) „Semi-targeted sequencing of fusion transcripts in prostate cancer enabled by oligonucleotide-modified dideoxynucleotides"

12-6-15 Dr. Virginija Dudutienė (Vilnius University, Lithuania) „Ligand augmentation in the active site of carbonic anhydrase leads to the discovery of CAIX-selective inhibitors"

13-8-15 Dr. Erki Enkvist (University of Tartu, Estonia) „Protein induced phosphorescence and its amplification throught the energy transfer"

14-13-16 Dr. Hajar Nika Estiri (Latvian Institute of Organic Synthesis, Latvia) „Epileptic seizure suppression by xenograft of engineered human Wharton's jelly mesenchymal stem cells in kindling model"

15-5-15/17 Marius Gedgaudas (Vilnius University, Lithuania) „*Thermott*: tool for protein stability and protein-ligand interaction analysis"

16-10-15 Dr. Renu Geetha Bai (Estonian University of Life Sciences, Estonia) „Production of microalgae for fish feed using flue gas as an input"

17-7-15/17 Aurimas Greičius (Institute of Biosciences, Lithuania) „Activity and functionality analysis of *Streptomyces* and *Arthrobacter* bacterial cutinases"

18-9-15/17 Henri Ingelman (University of Tartu, Estonia) „Faster growth enhances low carbon fuel and chemical production through gas fermentation"

19-11-15/17 Anatolii Ivankov (Vytautas Magnus University, Lithuania) „Changes in hemp growth and content of cannabinoids after seeds treatment with cold plasma, vacuum, and electromagnetic field"

20-15-16 Silvija Jankeviciute (Lithuanian University of Health Sciences, Lithuania) „The effect of metformin on cultured microglia cells under normoxic and mild-hypoxic conditions"

21-4-16/17 Eva Lea Jääger (University of Tartu, Estonia) „The development of a selenophene- and deazapurine-based photoluminescent probe of protein kinases"

22-13-15/17 Karl Jürgenstein (University of Tartu, Estonia)
„Translational fidelity in the absence of Ψ32 and Ψ38-40 in the anticodon stem-loop of tRNAs"

23-6-16/17 Justina Kamarauskaite (Lithuanian University of Health Sciences, Lithuania) „Ischemia/reperfusion and caffeic acid phenethyl ester effect on kidney mitochondria *in vivo*"

24-17-16 Dr. Sergei Kopanchuk (University of Tartu, Estonia) „(Sweet)MultiBac(Mam) library for frizzled receptors studies with fluorescence spectroscopy and microscopy"

25-8-16/17 Ksenija Korotkaja (Latvian Biomedical Research and Study Centre, Latvia) „Quantification of alphaviral vectors using droplet digital PCR"

26-12-15 Dr. Kaia Kukk (Latvian Biomedical Research and Study Centre, Latvia) „Expression of „the blue gene", flavonoid 3', 5'-hydroxylase from *Vaccinium* species in the yeast *Pichia pastoris*"

27-14-15 Dr. Maria Kulp (Tallinn University of Technology, Estonia) „Quantification of beta lactam antibiotics in human plasma by HPLC-MS/MS method for therapeutic drug monitoring"

28-10-16/17 Pille-Riin Laanet (Tallinn University of Technology, Estonia) „Antioxidative and anti-*Borrelia* activity of phytochemicals in *Rubiaceae* species"

29-15-15/17 Marlen Leemet (Tallinn University of Technology, Estonia) „The second life of mandarine pomace: a valuable source of bioactive compounds"

30-19-16 Dr. Ilva Liekniņa (Latvian Biomedical Research and Study Centre, Latvia) „Creation of virus-like particle-based therapeutic vaccine candidate against Alzheimer's disease"

31-17-15/17 Rebeka Ludviga (Latvian Biomedical Research and Study Centre, Latvia) „Marafivirus coat protein variants' expression in bacterial system for virus-like particle self-assembly"

32-16-15 Dr. Juliana Lukša (Vilnius University, Lithuania) „Impact of *Totiviridae* L-A dsRNA virus on *Saccharomyces cerevisiae* host: transcriptomic and proteomic approach"

33-21-16 Dr. Helike Lõhelaid (University of Helsinki, Finland) „Modulating microglia phagocytosis"

34-19-15/17 Greta Mačiuitytė (Vilnius University, Lithuania) „Oxyfunctionalization study of aromatic compounds by biocatalytic and chemical methods“

35-23-15/17 Vilius Malūnavičius (Vilnius University, Lithuania) „*Geobacillus* sp. 95 esterase: analysis of important amino acids for substrate binding and activity“

36-25-15/17 Bernadeta Masiulionytė (Vilnius University, Lithuania) „Engineered DNA methyltransferase DNMT1 for selective covalent tagging of methylation sites in live cells“

37-27-15/17 Domas Mašonis (Vilnius University, Lithuania) „Investigation of genes and enzymes involved in the metabolism of pyrazine derivatives“

38-12-16/17 Sten Miller (National Institute of Chemical Physics and Biophysics, Estonia) „Optimization and application of stable isotope mass spectrometry method to study breast cancer energy metabolism“

39-21-15/17 Marcel Mäger (Tallinn University of Technology, Estonia) „The biochemical characterization of *Thermus thermophilus* large laccase in relation to the valorization of lignin“

40-18-15 Deimantė Narauskaitė (Lithuanian University of Health Sciences, Lithuania) „Microglia immunometabolism changes after virus mimetic poly I:C-primed human airway exosome-like vesicles uptake“

41-20-15 Dr. **Zita Naučienė** (Vytautas Magnus University, Lithuania) „Pre-sowing treatment of common buckwheat seeds with cold plasma and electromagnetic field results in stimulated seedling growth and increased grain harvest“

42-14-16/17 Helvijs Niedra (Latvian Biomedical Research and Study Centre, Latvia) „Genome wide analysis of circulating miRNAs in growth hormone secreting pituitary neuroendocrine tumor patients' plasma“

43-29-15/17 Anete Ogriņa (Latvian Biomedical Research and Study Centre, Latvia) „Bacterial expression systems based on potato virus Y-like particles for vaccine generation“

44-23-16 Aistė Polikaitytė (Thermo Fisher Scientific Baltics UAB, Lithuania) „Detection of SARS-CoV-2 in clinical samples in <10 min using SuperScript IV RT-LAMP Master Mix“

45-16-16/17 Leenu Reinsalu (National Institute of Chemical Physics and Biophysics, Estonia) „Metabolic plasticity supports colorectal cancer progression"

46-25-16 Evelina Rekuviene (Lithuanian University of Health Sciences, Lithuania) „The effects of Complex I inhibitors on mitochondrial permeability transition and ischemic brain injury"

47-18-16/17 Oļesja Rogoza (Latvian Biomedical Research and Study Centre, Latvia) „Transcriptome study of pancreatic neuroendocrine tumour tissue"

48-20-16/17 Kristine Roos (Tallinn University of Technology, Estonia) „Using deconvolution to dissect cell-cluster-specific transcriptome profiles of the human preovulatory follicles to reveal gene expression disturbances between hypo- and normoresponders"

49-27-16 Dr. Evita Rostoka (University of Latvia, Latvia) „In search of biomarkers for type 1 diabetes"

50-22-15 Prof. Nils Rostoks (University of Latvia, Latvia) „Towards improving adaptability and resilience of perennial ryegrass for safe and sustainable food systems using genome editing – EditGrass4Food"

51-24-15 Anastasija Rudnickiha (LOSI, Latvia) „Optimization of crystallization conditions for stabilized tubulin complex"

52-26-15/52-44-17 Lukas Krasauskas (Vilnius University, Lithuania) „Pro-inflammatory S100A9 protein effect on Tau protein aggregation"

53-22-16/17 Naatan Seeba (Tallinn University of Technology, Estonia) „The effect of vegan diet on exogenic lipoprotein lipase activity in healthy individuals"

54-24-16 Dr. Kaido Viht (University of Tartu, Estonia) „Development of protein kinase inhibitors that lose affinity upon physical or chemical action"

55-29-16 Dr. Sandrita Simonyte (Lithuanian University of Health Sciences, Lithuania) „The effect of *Elsholtzia ciliata* essential oil on the oxidative stress in mice organs"

56-26-16/17 Misela Sivaka (Latvian Biomedical Research and Study Centre, Latvia) „Interaction and dynamics of intestinal IgA and gut microbiome profile during antidiabetic therapy"

57-31-16 Kristina Skemiene (Lithuanian University of Health Sciences, Lithuania) „The effects of imeglimin on developing brain microglial cells after hypoxia and reoxygenation"

58-33-16 Prof. Andrejs Skesters (Riga Stradins University, Latvia) „Are the course and severity of COVID-19 related to selenium, selenoprotein P and blood oxidative stress level in patients with acute and post-COVID-19 disease?"

59-33-15/17 Gerda Skinderytė (Vilnius University, Lithuania) „Insights of ScV-LBC Gag expression in *Saccharomyces cerevisiae* strains"

60-28-16/17 Giedrė Skliutė (Vilnius University, Lithuania) „Changes of immune response related genes expression profile in endometrium tissue of females with reproductive disorders"

61-35-15/17 Joana Smirnovienė (Vilnius University, Lithuania) „Engineering of chimeric carbonic anhydrases for designing isoform-selective inhibitors"

62-37-15/17 Ramunė Stanevičienė (Nature Research Centre, Lithuania) „Antagonistic traits of yeasts isolated from the surface of sour and sweet cherries"

63-39-15/17 Rokas Statkevičius (Vilnius University, Lithuania) „Aminotransferases for chiral amine synthesis"

64-30-16/17 Arvydas Strazdauskas (Lithuanian University of Health Sciences, Lithuania) „Ischemia *in vivo* causes cardiolipin peroxidation and mitochondrial damage in rat kidneys"

65-35-16 Dr. Rasa Sukackaitė (Thermo Fisher Scientific Baltics UAB, Lithuania) „MuA-based molecular indexing for rare mutation detection by next-generation sequencing"

66-31-15/17 Jelizaveta Širokova (Daugavpils University, Latvia) „Applications of new fluorescent disubstituted benzanthrone dyes for DNA study"

67-40-16/17 Vilma Zigmantaite (Lithuanian University of Health Sciences, Lithuania) „Effect of pro-antiarrhythmic *Elholtzia ciliata* essential oil on blood parameters in swine"

68-42-16/17 Deimantė Žukauskaitė (Vilnius University, Lithuania) „Different inducers impact for endometrial stromal cells *in vitro* decidualization"

69-32-16/17 Rutt Taba (National Institute of Chemical Physics and Biophysics, Estonia) „Effect of cell culture conditions on cellular bioenergetics and viability"

70-28-15 Dr. Paulius Lukas Tamošiūnas (Latvian Biomedical Research and Study Centre, Latvia) „Yeast-based system for *in vivo* evaluation of alleles of the anthocyanin production pathway"

71-41-15/17 Kannan Thirumalmuthu (Tallinn University of Technology, Estonia) „Organosolv lignin degradation by *Actinomycetales*"

72-43-15/17 Elisabeth Undrits (University of Tartu, Estonia) „Development of visible-light activated photocaged bisubstrate inhibitor of protein kinases"

73-30-15 Prof. Asko Uri (University of Tartu, Estonia) „Discovery of strong inhibitory properties of a monoclonal antibody of protein kinase A"

74-34-16/17 Maija Ustinova (Latvian Biomedical Research and Study Centre, Latvia) „Metagenomic characterization of untreated wastewater acquired from 15 municipalities of Latvia"

75-36-16/17 Ieva Vaicekauskaitė (National Cancer Institute, Lithuania) „Prognostic insights from the analysis of uterine lavage samples in ovarian cancer patients"

76-38-16/17 Algirdas Valys (Nature Research Centre, Lithuania) „Antimicrobial potency of essential oils"

77-37-16 Dr. Agne Velthut-Meikas (Tallinn University of Technology, Estonia) „Ovarian sensitivity index as a measure for female infertility correlates with endocrine disruptive chemical mixture exposure"

ABSTRACTS

PLENARY SESSION

FEBS – ADVANCING MOLECULAR SCIENCES

J. Dumić

University of Zagreb Faculty of Pharmacy and Biochemistry
FEBS Executive Committee / FEBS Working Group on Integration

The Federation of European Biochemical Societies (FEBS) is a charitable organisation that supports research and education in molecular life sciences across Europe and in neighboring countries. Founded in 1964, FEBS has become one of Europe's largest organizations in the molecular life sciences, with over 35,000 members, who belong to 38 constituent Societies. The main object of the Federation is to contribute to and promote the advancement of research and education for the public benefit in the sciences of biochemistry and molecular biology and related disciplines through its journals, Congress, Advanced Courses, Fellowships, and other initiatives. In the presentation activities and initiatives of the FEBS will be presented in more detail. For further information, please visit www.febs.org.

CLINICAL PROTEOMICS QUO VADIS? FINE-TUNING CLINICAL PROTEOMICS FOR THE FUTURE

M. H. Baumann

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In the year 2002 Carol Ezzell wrote in the *Scientific American* (1) an article about the importance of proteins after the publication of the initial rough draft of the human genome in February 2001. He stated that proteomics is the biotech's next big challenge. The most optimistic scientists where in the believe that the human proteome would be successfully mapped in three years, depending on how you define „Proteome". In 2011 the journal *Drug Discovery and Development* wrote on the front page that there was still a long way to the bedside and that despite many breakthroughs proteomics had not translated yet to the patient care. Today, 11 years later we can still witness the ongoing „developmental phase" in modern proteomics intended for clinical use. It has become evident that commonly encountered shortcomings in fundamental aspects of experimental design in clinical proteomics and the still existing limitations of the current technology must be addressed in order to provide the final breakthrough for the utilization of proteomics in the everyday clinical environment. Luckily, we are soon supported by proteomics techniques of the future, not solely based on classical approaches, such as mass spectrometers or antibody-based technologies, helping to take the final step for modern clinical proteomics. Companies like Quantum SI, Nautilus Biotechnology, Eriyson, Encodia, Alamar Biosciences, Quanterix, Seer Inc, Biodesix and others will have a substantial impact on how we perform clinical proteomics in the future.

References

1. https://www.jstor.org/stable/pdf/26059640.pdf.

FLUORESCENT PROBES FOR CAIX EXPRESSED IN LIVE CANCER CELLS

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Human cancers express carbonic anhydrase IX (CAIX), a transmembrane protein that acidifies tumor microenvironment, promotes metastases, and is considered a promising anticancer target. We have designed high affinity and specificity fluorescently-labeled CAIX inhibitors to visualize and quantify CAIX expression in cancer cells. We applied a competitive binding model to determine compound dissociation constants for CAIX expressed in cancer cells. The sulfonamide compounds bound to proliferating cells with same affinity as to purified CAIX catalytic domain. The probes are applicable for the design of selective compounds for CAIX, while the competition strategy could be applied to medicinal chemistry of various drug targets.

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STRUCTURAL ANALYSIS OF AN ANTIGEN CHEMICALLY COUPLED ON VIRUS-LIKE PARTICLES

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Structural analysis of adjuvant-coupled antigens is important for rational vaccine development but has been impeded by the lack of appropriate techniques. In principle, magic-angle spinning (MAS) solid-state NMR is pertinent to the study of such dynamic and heterogeneous systems, however its application has been hampered by the relatively low antigen content in vaccine formulations. We will show that sensitivity enhancement provided by high-field dynamic nuclear polarization (DNP) and proton detection at fast MAS rates allows to overcome the penalty associated with the antigen dilution. This makes it possible to assess the structure of the influenza virus hemagglutinin stalk long alpha helix antigen, both in its free, unformulated form and once chemically coupled to the surface of large virus-like particles (VLPs). Comparison of the MAS NMR fingerprints between the free and VLP-coupled forms of the antigen provides site-specific information of the structural change or conservation occurring upon bioconjugation. Thereby, this work[1] demonstrates that high-sensitivity MAS NMR is ripe to play a major role in vaccine design, formulation studies, and manufacturing process development.

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HUMAN DRUG TARGETS: TRENDS IN DRUG DISCOVERY

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The talk will focus on the identity of drug targets derived from the human genome. Investigations on drug targets for FDA approved agents and drugs in clinical trials has provided information on the trends within drugs discovery. Drug targeting has undergone major changes because of new opportunities to target drugs with different types of biotechnologically derived drugs. Monoclonal antibodies, new recombinant proteins and new synthetic peptides stand for a very large portion of agents in clinical trials. The diversity of agents is expanding, including vaccines aimed at producing an immune response to endogenous pathogenic proteins, and nucleic acid therapies such as antisense oligonucleotides and small interfering RNAs (siRNAs) for modulating gene expression. Membrane bound proteins including many receptors, transporters and enzyme families are major classes for drug discovery; and G protein-coupled receptors (GPCRs) form the largest set of drug targets within the human genome. The talk will cover the trends in kinase drug discovery considering targets (Attwood et al., *Nature Reviews Drug Discovery*, 2021), indications and inhibitor design. Moreover, soluble ligands as drug targets (Attwood et al., *Nature Reviews Drug Discovery*, 2020) and brain cancer drug discovery (Sokolov et al., *Pharmacol Rev.* 2021) will also be addressed.

SESSION 2

BIRTH OF A PROTEIN: FROM DEVELOPMENT TO PRODUCTION

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Heterologous protein expression is an invaluable tool for both research and biotech industry. The most common host for recombinant protein production is *E. coli*. In Solis BioDyne we have successfully applied *E. coli* for the production of DNA polymerases, reverse transcriptases, an uracil-DNA-glycosylase and a RNase inhibitor. All our proteins are expressed with a Stability Tag – a patented technology that increases polypeptide shelf life and its tolerance for temperature at a wider scale without compromising the properties of the polypeptide itself.[1,2] Our products are combined into different mixes for PCR, qPCR and cDNA synthesis.[2] Thanks to our previous experience in the development of enzyme mixes we were able to offer a kit for the detection of SARS-CoV-2 as early as in spring 2020. The kit has been valued by customers worldwide. Furthermore, the great demand of biomolecular reagents during the coronavirus pandemic has forced us to rapidly upscale our production. The current presentation points out some of the difficulties we have experienced and changes in the purification workflow that we have made when stepping from the development to upscaled protein production. Moreover, the optimization of protein production as well as the development of new proteins that would be applicable for current methods is an ongoing process.

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INHALATION BASED DELIVERY OF BROADLY NEUTRALIZING SARS-COV-2 ANTIBODIES

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Icosagen established the development of SARS-CoV-2 neutralizing antibodies in the light of the 2020 SARS-CoV-2 global pandemic. Taking advantage of our established QMCF and HybriFree technologies and workflows, we were rapidly able to develop highly potent SARS-CoV-2 neutralizing antibodies. As the viral antigenic landscape has been changing with the emergence of different variants of concern (VoC), many initially developed virus neutralizing antibodies have proven to be ineffective against current Omicron variants. Here we demonstrate the development of a pan-VoC neutralizing humanized SARS-CoV-2 Spike antibody. Furthermore, to overcome the cumbersome intravenous administration of such antibody based therapeutics we have established an inhalation based delivery approach of SARS-CoV-2 neutralizing antibodies and demonstrate in-vivo efficacy in non-human primate models.

POTENTIOMETRY FOR STUDIES OF MEMBRANES AND ENERGETICS IN MICROORGANISMS

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Most biochemists use potentiometry as a research method, but only for measurement pH of the solutions. However, potentiometric analysis using ion-selective electrodes can be employed for getting more various information on biological micro-objects, such as bacteria, yeast cells, or isolated mitochondria. Even monitoring the pH of microbial suspensions we can get information on the intensity of energy metabolism of cells or detect changes in the permeability of membranes. Electrochemical monitoring of the activity of microorganisms allows the additional analyses of the samples taken directly from the vessels for electrochemical measurements, for example, analysis of the absorbance or fluorescence, determinations of ATP content, or counting of the viable cells or the infectious virus particles. During the potentiometric analysis, aeration of the cell suspensions can be controlled, the incubation vessels can be thermostated. In my talk I will analyze how the potentiometric measurements using selective electrodes could be used for determination of the physiological activity and/or the viability of cells, assays of the sensitivity of studied microorganisms to antimicrobial compounds. Examples will be presented, how the sensitivity of bacteria to bacteriophages, duration of the infection cycles can be electrochemically monitored, or the resistance of bacteria to antibiotics can be determined. Potentiometric analysis of the energy metabolism in microbial suspensions and search for the inhibitors of efflux pumps are another research areas to be discussed. Principles of construction of PVC membrane-based ion-selective electrodes for assays of the indicator ions will also be discussed.

A COMPREHENSIVE TOOL FOR PROTEIN-LIGAND BINDING CONSTANT DETERMINATION BY THERMAL SHIFT ASSAY

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The thermal shift assay is a universal technique to determine protein-ligand affinities ranging from millimolar to picomolar levels. Widely applicable thermal shift assay experiments in various forms have been performed for many years. However, the protein-ligand dissociation constant was rarely calculated due to the lack of a suitable data analysis tool. Here we present a significant advancement to the analysis of protein-ligand binding data obtained by the thermal shift assay. Our new software *Thermott* is an open-source, online data analysis tool providing an easy-to-use nonlinear regression analysis of complex thermodynamic equations[1]. The synergy of widely accessible experimental thermal shift assay and the new analysis tool could expand the use of this technique in drug discovery and other biotechnology fields.

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LIVE CELL FLUORESCENCE-BASED LIGAND BINDING ASSAYS POWERED BY AUTOMATED MICROSCOPY AND MACHINE LEARNING IMAGE ANALYSIS

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Live-cell microscopy holds a key position in pharmacology as it provides rich spatial information without significant sacrifice in temporal resolution or cost. Despite its usefulness, quantification of microscopy images has

been difficult due to a large amount of data and unreliable image analysis algorithms, but recent advances in computer vision can solve this problem. The deep convolutional neural networks are a powerful subset of machine learning algorithms which can be used for detecting, segmenting and classifying objects from images among other tasks.

To showcase the possibilities of combining live-cell microscopy with deep learning (DL) models, we used novel fluorescence ligands, NAPS-Cy3B and UR-CG072, for dopamine D3 receptors expressed in HEK-293 cells and for muscarinic acetylcholine M4 receptors expressed in CHO-K1 cells, respectively, for ligand binding assay development.

We used a user-friendly yet less powerful Ilasik based random forest algorithm for cell contour segmentation from bright-field images in the case of HEK-293-D3R. The model provided high-quality performance, which had a substantial advantage over the average image intensity calculation method. we used the assay for measuring the binding parameters of fluorescence ligands as well as unlabeled ligands to D3 receptors.

Further, we compared the quality and performance of Ilastik based random forest segmenter and a DL architecture U-Net and validated both for quantifying fluorescent ligand binding to M4 receptors. In general, both models work, but U-Net based solution offers higher quality results along with faster imaging and higher image processing speed.

In live-cell microscopy, different anomalies are often present, which affect image analysis quality. As imaging artefacts such as dead cells and foreign objects have a much larger variability of shape and structure than cells then weakly supervised methods are more practical for solving this problem. For anomaly removal, we developed a ScoreCAM-U-Net model that achieved almost human-level quality in a fraction of time.

We have combined the developed models with the *MembraneTools* module of *Aparecium* software (https://gpcr.ut.ee/aparecium.html) for the convenient application of these algorithms.

SESSION 3

ENZYMATIC FIBRE MODIFICATIONS: FROM FUNDAMENTALS TO APPLICATIONS

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Cellulosic fibres have broad application range from paper and packaging, hygiene products and textiles to food, feed, cosmetics and medicine. Enzymatic catalysis enables specific modifications of fibre polysaccharides and sustainable processing technologies for conversion of cellulosic fibres into material components. Cellulases (cellobiohydrolases (CBHs), endoglucanases (EGs)) and lytic polysaccharide monooxygenases (LPMOs) catalyze hydrolytic and oxidative reactions in cellulose polymers, respectively. CBHs hydrolyze cellulose crystals in processive manner, while EGs catalyze hydrolysis of internal glycosidic linkages in non-ordered areas. LPMOs catalyze hydroxylation at C1 and/or dehydrogenation at C4 position of anhydroglucose units in crystalline cellulose, which leads to a cleavage of the glycosidic linkages and formation of δ-1,5 lactone or 4-ketoaldose end groups. Treatment of cellulosic fibres at high fibre consistency (≥20 % dry matter) with CBH-rich enzymes induced a peeling-type of fibrillation and produced a paste-like material, suitable for e.g. coatings[1]. Treatment of fibres with EGs at high fibre consistency strongly reduced the cellulose molar mass and improved fibre reactivity toward dissolving chemicals, beneficial in production of regenerated textile fibres and cellulose derivatives[2]. Oxidative disruption of the cellulosic fibres with LPMOs also improved fibre nanofibrillation and solubility, but leaved cellulose molar mass higher compared to EGs.[3,4] The fundamental differences in enzyme-catalyzed molecular level modifications of the cellulose polymers thus translate into distinct changes in macroscopic fibre properties, and enable processing of the fibres into different types of material components.

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H_2O_2 COSUBSTRATE IS A DOUBLE-EDGED SWORD FOR LYTIC POLYSACCHARIDE MONOOXYGENASES (LPMOs)

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Because of their ability to break glycosidic bonds in recalcitrant polysaccharides like cellulose and chitin, the catalysis by lytic polysaccharide monooxygenases (LPMOs) is of major interest. These monocopper enzymes depend on the H_2O_2 cosubstrate and the presence of the reductant. In polysaccharide peroxygenase reaction the H_2O_2 is consumed stoichiometrically with glycosidic bond cleavage, while reductant is needed only for initial „priming" of LPMO-Cu(II) resting state to its catalytically active LPMO-Cu(I) form. However, polysaccharide free LPMO-Cu(I) is amenable to reoxidation by O_2 (reductant oxidase reaction) and H_2O_2 (reductant peroxidase reaction). Unfortunately, the reoxidation by H_2O_2 may lead to the irreversible inactivation of the enzyme. This unwanted side reaction is a major drawback in industrial application of these powerful enzymes. During last 5 years we have performed kinetic characterization of the H_2O_2-driven catalysis by multiple LPMOs of both bacterial and fungal origin. On the applied side of LPMO science, we have shown that enzyme-independent redox reactions in industrially relevant streams of lignocellulose biorefining may lead to the accumulation of H_2O_2 to the levels that are detrimental for LPMOs. We hope that an in-depth understanding of the kinetics of different LPMO catalyzed reactions along with the enzyme-independent reactions that influence the dynamics of H_2O_2, helps knowledge-based adjusting of the reaction conditions for optimal stability of LPMOs.

ANTIMICROBIAL MATERIALS AND SURFACE COATINGS: AN EFFECTIVE SOLUTION OR A MATTER OF CONCERN

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The growing interest towards the use of antimicrobials, especially non-antibiotic antimicrobials, for hygiene purposes has been evidenced by the increased level of research publications as well as by steady increase of their market share. There is an increasing trend towards the inclusion of antimicrobial properties into various materials and surface coatings that usually have a relatively lower but sustained antimicrobial effect compared with traditional biocidal disinfectants. A wide variety of antimicrobials, ranging from metals to quaternary ammonium compounds and antibiotics, can be used to produce such antimicrobial surface coatings and their area of use ranges from biomedicine to construction industry.

Antimicrobial compound-based surfaces usually act by releasing the antibacterial active agent thereby inactivating both, microbial cells on and around surfaces, or in situ, killing only surface-deposited microbes. Two main properties are expected from such surfaces: activity against the target microbes and safety against other, non-target species. Theoretically, the antimicrobial activity should be guaranteed by regulatory requirements for antimicrobial coatings. However, the current regulation foresees only testing of antimicrobial surface coatings in standardized conditions and does not require a proof of their efficacy in real-life imitating conditions. The work towards optimizing test protocols for real-life like testing condition for antimicrobial materials is one of the focuses of our research group and examples of such optimizations will be discussed in the presentation. Similar to the efficacy, the existing regulations should also ensure the safety of antimicrobial materials and coatings. However, although the chemical safety of antimicrobials is usually addressed, recent concerns have been raised regarding the potential of such materials to induce antimicrobial resistance in human-related and natural communities. Indeed, there is evidence that metal-containing antimicrobial compounds have increased the level of metal resistance in natural communities and that resistance to metals links with that of antibiotics, often in similar genetic elements. In the presentation, three possible examples of co-resistance development towards known

antimicrobial compounds (QAC, copper and silver) and antibiotics, will be discussed. Finally, the pros and cons of using antimicrobial materials and surfaces as means to improve public health, will be discussed.

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UNRAVELING ACETOGEN GAS FERMENTATION USING QUANTITATIVE SYSTEMS BIOLOGY

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The world faces an increasing need for sustainable production of fuels and chemicals. Recently, acetogens have gained significance as cell factories for converting inexpensive and abundant waste feedstocks (e.g. syngas [CO, H_2, CO_2], industrial waste gases) into high-value products using gas fermentation. Furthermore, acetogens are ancient microorganisms as they arguably use the first carbon fixation pathway on Earth to fix CO_2. Although biochemistry of the Wood-Ljungdahl pathway is well understood, better understanding of acetogen metabolism is needed for their rational metabolic engineering. We have developed a systems biology platform for advancing the understanding and engineering of acetogen metabolism through unravelling regulatory features and building the first quantitative links between carbon, energy, and redox metabolism. We used steady-state autotrophic chemostat cultivation coupled to quantitative gas,

transcriptomics, proteomics, metabolomics analyses to reconstruct acetogen metabolism *in silico* using genome-scale metabolic models and thermodynamic metabolic flux analysis. This systems-level approach led us to propose a novel regulatory mechanism for carbon distribution and factors limiting metabolic robustness in acetogens. Metabolic modelling could accurately predict growth phenotypes and absolute proteomics data quantified allocation of protein resources in the model-acetogen *Clostridium autoethanogenum*. Coupling flux and proteomics data identified the prevalence of post-translational regulation of metabolic fluxes by adjustment of apparent *in vivo* catalytic rates of enzymes. We further used differential RNA-sequencing to decipher transcriptional architecture and to identify a key novel promoter motif together with a transcriptional regulator associated with essential genes for autotrophic growth of acetogens. Our work both serves as a reference dataset and advances fundamental understanding of regulation in the ancient metabolism of acetogens. It also suggests strategies for improving the performance of gas-fermenting acetogens through rational metabolic engineering.

DEVELOPING LOCALLY APPLICABLE BIOTECHNOLOGY VALUE CHAINS

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Non-conventional yeast *Rhodutorula toruloides* has many advantages in front of traditional model organisms when desired to be used as a microbial cell factory for converting various waste into value added products like food ingredients. *R. toruloides* can naturally consume a variety of carbon sources, have high stress tolerance towards fluctuations in environmental conditions and toxic compounds, and is naturally producing a number of valuable food ingredients, including lipids (up to 70% of their biomass) and carotenoids. However, to make this strain more efficient and allow the production of value-added nutrients, the strain's metabolism must be well understood and efficient tools for its metabolic engineering must be developed.

In order to do so, we have sequenced the strain, evolved it to tolerate high concentration of industrial hydrolysates, developed advanced metabolic modeling tools alongside with the development of a synthetic biology toolbox for the yeast to allow an efficient engineering of the strain. Within this framework, we have learned peculiarities of co-factor balancing and energy metabolism of this oleaginous yeast. In this presentation, we exemplify how the advanced modelling of *R. toruloides* have helped us to improve its uptake of hemicellulosic carbon sources and increase the efficiency of converting them into natural and unnatural carotenoids.

SESSION 4

ER STRESS REGULATING CDNF AND MANF PROTEINS PROTECT CELLS BY NOVEL MECHANISM

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In Parkinson's disease (PD) midbrain dopamine (DA) neurons degenerate and die causing in addition to major motor symptoms also non-motor symptoms. Current drugs can only temporarily alleviate the motor symptoms, but non-motor symptoms remain untreated. Our group has discovered an endoplasmic reticulum (ER) located protein with neurotrophic factor (NTF) activities – cerebral dopamine neurotrophic factors (CDNF). We have solved the three-dimensional structure of CDNF and its homologous protein MANF and found that their structure and mode of action radically differs from other known NTFs. CDNF and MANF are known to regulate unfolded protein response (UPR), signalling machinery aimed to restore cellular homeostasis upon increased loading of ER with misfolded or aggregated proteins. UPR is occurring in PD and many other pathological conditions. Aggregated α-synuclein is the main component of Levy bodies and CDNF reduces its aggregation and spreading and alleviates behavioural alterations in animal models of PD. The exact mechanism of how CDNF and MANF regulate UPR is unknown and receptors for CDNF and MANF have not been found yet. Using computational modelling, site-directed mutagenesis, and binding studies, we have identified putative ER-located receptors of CDNF and MANF. We are currently investigating the physiological implications of CDNF and MANF interactions with their receptor.

We have demonstrated that CDNF can protect and repair midbrain DA neurons in rodent neurotoxin models of PD at least as efficiently as other known NTFs. CDNF was more efficient than GDNF in rhesus monkey neurotoxin model of PD. Herantis Pharma Plc. has tested CDNF in phase I-II clinical trials in PD patients and CDNF achieved its primary endpoint of safety and tolerability. Moreover, significant increases in DAT PET signalling

and improved UPDRS scores were observed in some, but not all, CDNF-treated patients.

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EXTRACELLULAR VESICLES AS INFLAMMATORY MEDIATORS BETWEEN THE AIRWAY AND BRAIN

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Viral respiratory infections induce airway cell extracellular vesicles (EVs) containing viral genetic material and inflammatory mediators. Exosomes remain stable in biofluids, penetrate well into the tissues, and easily cross biological barriers, including the blood-brain barrier. Therefore, they can transmit the inflammatory signal to the brain; however, the hypothesis has not been experimentally tested. The study aimed to determine whether virus mimicking sequence poly I:C-primed airway EVs enter the brain and, if yes, how they might affect the inflammatory status of the brain cells.

EVs from poly I:C-treated airway cell culture medium were isolated by polymer precipitation/centrifugation, characterised by particle number/size, morphology, and specific markers. For *in vivo* and *in vitro* EV tracking, they were fluorescently labelled, and the viral material transfer was monitored using DHR-poly I:C. Mitochondria and glycolysis were assessed by a Seahorse analyser. Intracellular ROS were determined by DCFDH, mitochondrial – by MitoSOX™ Red. Inflammasome activation was detected by the caspase-1 luminescent assay. Inflammatory gene expression was assessed by RT-PCR.

Poly I:C-primed airway EVs entered the brain within an hour after intranasal delivery and localised primarily in microglial cells. The EVs internalised poly I:C molecules and caused inflammatory immunometabolic profile of microglial cells, including energy production switch from mitochondrial to glycolytic, generation of reactive oxygen species and inflammasome-related caspase-1 activation. The EVs significantly elevated the expression of inflammatory genes in cultured primary rat microglia, human microglial cells and the brain tissue of EV-treated mice.

In conclusion, EVs from virus-infected airway cells might transmit viral particles and inflammatory signalling to the brain via microglial cells.

HOW KNOWLEDGE OF BIOCHEMISTRY CAN HELP CLINICAL MEDICINE: AN EXAMPLE OF REPRODUCTIVE MEDICINE

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Human reproduction is as complex as any other biological process. However, the fact that two healthy partners are needed to have a child makes it challenging. Therefore, the bottlenecks of reproductive medicine are wide-ranging, covering the preconception health of both partners, and fertility of men and women, the maturation of gametes, fertilization of the oocyte, the development and implantation of an embryo, and the growth of pregnancy until the birth of a healthy child. Thus, fertility is not only in the 9 months of pregnancy but it begins when the woman or man herself is a fetus. Because of the complexity of the reproductive process, there are many opportunities for this process to be disrupted, which can result in medical problems that prevent the couple from becoming pregnant, result in pregnancy loss, having a severe maternal illness during the pregnancy, or giving birth to a sick child. Therefore, reproductive medicine tries to solve many problems that prevent the birth of a healthy child. Based on

the new accumulating knowledge, we are already much better able to advise families before pregnancy, during the pregnancy and after the birth of a child. Infertility, which is caused by the infertility of a woman or a man, or both, accounts for a large proportion of reproductive problems. Infertility is most commonly treated by using in vitro fertilization (IVF), resulting in up to 10% of newborns being conceived in Europe. The topic of this presentation deals with the development and implementation of innovative genomic and biochemistry solutions into the diagnosis of infertility and in the treatment of infertility both in Estonia and globally.

FUNCTIONAL ROLES OF EXERCISE-INDUCED EXTRACELLULAR VESICLES IN THE PREVENTION OF CANCER

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Several lines of evidence suggest that regular physical exercise not only reduces the risk of cancer but also improves functional capacity, treatment efficacy and disease outcome in cancer patients. Recent studies have shown that extracellular vesicles (EVs) are released into the circulation during physical activity. We hypothesized that exercise-induced EVs may directly interact with cancer cells and alter their behavior and/or change the functional phenotype of circulating and tumor-infiltrating immune cells.

At first, we investigated the effects of exercise-induced EVs on the progression of cancer in a rat model of metastatic prostate cancer. Plasma samples were collected before and after the exercise from rats subjected to regular forced wheel running exercise and sedentary rats. EVs were isolated using SEC and characterized by TEM and NTA. RNA content of EVs was studied by RNA sequencing analysis. We did not observe a consistent increase in the circulating EV levels after the exercise, however the RNA sequencing analysis demonstrated substantial changes in the RNA

content of EVs collected before and immediately after forced wheel running exercise as well as differences between EVs from runners at resting state and sedentary rats. The major RNA biotype in EVs was mRNA, followed by miRNA and rRNA. Molecular functions of differentially expressed RNAs reflected various physiological processes including protein folding, metabolism and regulation of immune responses triggered by the exercise in the parental cells. Intravenous administration of exercise-induced EVs into F344 rats with orthotopically injected syngeneic prostate cancer cells PLS10, demonstrated reduction of the primary tumor volume by 35% and possibly – attenuation of lung metastases. Next, we investigated the RNA content of circulating EVs collected before and after a physical exercise from 10 healthy female runners and studied their uptake and the effects on transcriptional landscape in MCF7 and MDA-MB-231 breast cancer cells. Here, we will present the first results of this study.

DEVELOPMENT OF AN IN VIVO MODEL SYSTEM FOR CARBONIC ANHYDRASE 9 TARGETING IN BREAST CANCER

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Introduction: Carbonic anhydrase 9 (CAIX) is a cancer-associated membrane protein frequently overexpressed in hypoxic tumours leading to enhanced tumour cell survival and invasion. It has been proposed to be an attractive tumour-specific target. The aim of this study was to establish a mouse 4T1 breast cancer model that could model scenarios with constitutive CAIX expression or no expression of CAIX irrespective of the oxygen availability that could be further used for testing novel anti-CAIX targeting strategies.

Material and methods: The model system is based on the T41 model of triple-negative breast cancer in syngeneic Balb/c mice. 4T1 cells overproduce CAIX only in hypoxic conditions. By using lentiviral vector transfection system, we generated 4T1 cells that constitutively express

mCAIX under CMV promoter (4T1-*Car9*KI), and a cell line, in which the Car9 gene is inactivated by using CRISPR-Cas9 genome editing (4T1-*Car9* KO). Individual clones were selected, sequenced and mCAIX expression in hypoxic and normoxic conditions verified by Western blot. Three groups of adult Balb/c mice (n=7) were injected with 4T1 cells (7x103 in 50% Matrigel) in the third mammary fat pad. Group 1 received parental 4T1 cells, group 2 – 4T1-*Car9* KO cells, group 3 – 4T1-*Car9* KI cells. Tumour size was measured every 3 days.

Results: The results showed significantly slower tumour growth rates in the group 2 animals ($P < 0.002$). Group 1 and 3 animals with CAIX expressing 4T1 cells reached maximum tumour volume on day 25 while the group 2 animals – on day 39. The mCAIX production in tumours was verified by *in vivo* imaging using HypoxiSense 680 probe. Noteworthy, group 2 animals had normal spleen size while animals bearing CAIX producing tumours had splenomegaly (P=0.0004). No profound macrometastases formation was observed.

Conclusions: The developed syngeneic orthotopic mouse model provides full competence with regards to the natural tumour microenvironment and immunological responses, and provides adequate positive and negative controls for CAIX targeted therapy efficacy testing.

DETECTION OF SARS-COV-2 RNA IN WASTEWATER: IMPORTANCE OF VIRAL LINAGE AND POPULATION SIZE ASSESSMENT

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COVID-19 pandemic has highlighted the advantages of Wastewater-based epidemiology (WBE). However, the mobility of population, precipitation and irregular inflow of industrial wastewater are complicating the interpretation of acquired data, thus, providing additional challenges for assessment of disease prevalence and proper crisis management. These difficulties are particularly pronounced in less populated areas, because residents of such territories are more mobile and less strict in following the rules and guidelines of disease containment. Recent observations also suggest that the extent of fecal SARS CoV 2 shedding is linage specific, which adds an additional dimension of complexity to interpretation WBE results. Our studies demonstrate that combination of the data on detected SARS-CoV-2 RNA copy number in wastewater and such population size markers as wastewater 5-hydroxyindoleacetic acid (5-HIAA) or unique mobile phone caller records are important for accurate assessment of the COVID-19 epidemiological situation in small and medium-sized towns, while assessment of viral mutation repertoire can provide support when inconsistencies between WBE and data from clinical epidemiology based surveillance are detected.

NLRP3 INFLAMMASOME ACTIVATION BY IMMUNE COMPLEXES OF VIRUS-LIKE PARTICLES IN MICROGLIA

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The inflammasome is a vital 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 procaspase-1[1]. NLRP3 inflammasome activation results in cleavage and activation of inflammatory cytokines, like IL-1β, and inflammatory cell death – pyroptosis[2]. In our previous research we showed that viral proteins trigger 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 immune complexes composed of mouse immunoglobulins bound to virus-like particles (VLPs) of these human polyomaviruses: KI polyomavirus (PyV), MCPyV, WUPyV[4]. 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 immune complexes of VLPs induced cell death, IL-1β secretion and ASC speck formation in microglia indicating NLRP3 inflammasome activation. In addition, immune complexes mediated significantly higher cellular response compared to VLPs alone. To conclude, our results demonstrate that immune complexes can enhance inflammasome activation.

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IMMUNOLOGICAL COMPARISON OF RECOMBINANT SHRIMP ALLERGEN PEN M 4, PRODUCED IN PICHIA PASTORIS AND ESCHERICHIA COLI

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Shellfish is one of the leading causes of allergy worldwide, affecting up to 10.3% of the general population. Shrimp allergy is commonly diagnosed using naturally sourced allergen extracts; however, this approach is inherently problematic and can lead to a considerable variety in results[1]. The alternative is recombinant allergens, which offer component-resolved diagnosis and personalized treatment.

Pen m 4 represents a clinically relevant allergen in *Penaeus monodon* shrimp as it causes specific sensitization, does not cross-react with dust mite and cockroach allergens, and, in children, it is the leading cause of shrimp allergy[2].

The objectives of this research were designed: 1) to identify whether the production of recombinant Pen m 4 (rP4) in a eukaryotic expression system, *Pichia pastoris*, will impact diagnostic test sensitivity and accuracy compared to *Escherichia coli*-produced equivalents; 2) to determine whether fusion with the maltose-binding protein (MBP) would improve rP4 yield; 3) to estimate whether glycosylation affected rP4 recognition by specific IgE (sIgE).

This study has shown that fusion of rP4 with MBP improved protein yield 1.3 and 2.3 times in bacteria and yeast, respectively. Protein microarray assay evaluated the sIgE response to the synthesized rP4 proteins in the sera of

shrimp allergic patients. The results showed that *E. coli*-produced rP4 proteins demonstrated higher sensitivity than their *P. pastoris*-produced equivalents.

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SESSION 5A

A NEW MEDICINE IN YOUR PRESCRIPTION – MESSENGER RNA

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The technology behind messenger RNA, or mRNA, has been in development since it was discovered in 1960, but the Pfizer and Moderna Covid vaccines marked the first time it had been approved for use in humans. It's now being applied to the development of dosens different vaccines. It is expected that RNA therapeutics will comprise a group of drugs that will change the current treatment for many human diseases. In this presentation we will discuss general concepts of mRNA-based therapeutics: structural features, prophylactic vaccines, gene replacement and cancer therapy. We will list challenges and advantages associated with manufacturing and use of RNA-based drugs and overview future perspectives.

STEALTH BRUSH POLYMERS AVOID RAPID CLEARANCE AND DISPLAY EFFECTIVE ACCUMULATION IN TNBC TUMOURS

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Triple-negative breast cancer (TNBC) is a highly aggressive form of breast cancer with a poor short-term prognosis. It is treated with aggressive chemotherapy which is accompanied by severe side effects. Nanoparticles (NPs) have the potential to reduce the severity of the side effects by selective

delivery of therapeutics to tumours. Tumour selectivity by non-targeted NPs is often explained by the enhanced permeability-retention (EPR) effect. The full capacity of EPR and the optimal size for its exploitation remains unclear due to the rapid non-specific opsonization of NPs in plasma and associated changes in size and surface properties. Here, we prepared heavily PEGylated brush polymers with 18, 29 and 54 nm hydrodynamic diameters to study EPR of TNBC. Using fluorescence correlation spectroscopy we observed no changes in the sizes of brush polymers after one week of incubation in full mouse serum, attesting to their stealth nature and ability to resist non-specific opsonization. Brush polymers of all tested sizes demonstrated long circulation times (t1/2 exceeding 3 days) and broad biodistribution in healthy female Balb/c mice (2 weeks post-injection). Pharmacokinetic profiles were not affected significantly by the presence of 4T1 TNBC tumours; tumours, however, were the major accumulation sites for all tested materials. Tumour selectivity was inversely dependent on size, with liver/tumour ratios of 0.25, 0.40, and 0.76 for brush polymers with DH 18, 29, and 54 nm, respectively. Brush polymer concentrations in spleens of tumour-bearing mice were significantly (2.6-4.4 times) lower than in healthy counterparts, however, distribution to other organs was not affected by the presence of tumour. Overall, we demonstrated that using non-opsonizing nanoparticles can take advantage of EPR in TNBC, but tumour selectivity decreases with an increase in NP size.

ALTERNATIVE ENERGY: THE ROLE OF BRANCHED-CHAINED AMINO ACIDS IN BREAST CANCER CELLS

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It is known that cancer cells have specific metabolism. Degradation of branched chain amino acids (BCAAs) is one of the common pathway in different cancer cells. According our previous studies, branched-chained

amino acid transferase 2 (BCAT2), which breaks down BCAAs for energy production, is overexpressed in some breast cancer cell lines. However, the mechanisms of cancer cells growth support by the degradation of BCAAs remains uncertain. We performed 13C experiments in order to elucidate the metabolic role of BCAA degradation in breast cancer cell lines. In order to quantify the potential role of amino acids as sources affecting the total ATP amount, as well as labelling pattern of BCAA degradation products, Genome Scale Metabolic Model was used. The fractions of labelled citrate, malate, as well as mevalonate and mitochondrial acetyl-CoA were estimated. Our results suggest that simultaneous processes of lipid synthesis and oxidation can proceed in particular breast cancer cells. Higher fully labelled citric acid rate phenomenon was related to higher initial mitochondrial potential in breast cancer cells. Moreover, we predict that leucine is responsible for the supply of mitochondrial acetyl-CoA.

ACIDIFICATION OF BLOOD PLASMA FACILITATES THE ISOLATION AND ANALYSIS OF EXTRACELLULAR VESICLES

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Blood plasma is available with minimal invasive sampling, has significant diagnostic utility, and it is a valuable source of extracellular vesicles (EVs). Nevertheless, the complexity of such biofluid, and difficulties it poses on EVs isolation and analyses, is unanimously recognized in the research community. Rich protein content, presence of lipoproteins (LPs) that share similar biophysical properties, and relatively low abundance of EVs, especially those of rare subpopulations, make any downstream application a very challenging task. Growing evidence of EVs' intricate surface interactome, and their association to LPs, further aggravates their

purification, detection, and biomarker analyses. In this study, we tackled the fundamental issues of plasma EVs yield and LPs co-isolation, by applying moderate acidification of plasma in the upstream processing, to disrupt LPs and improve recovery of EVs. Our results demonstrate highly efficient enrichment of EVs' and partial depletion of LPs' markers when combining acidification with size exclusion chromatography (SEC) and/or differential centrifugation (DC). Furthermore, it enables quick single-step precipitation of up to 50% of EVs directly from plasma, upon short low-speed centrifugation. Acidification holds potential as a simple, effective, and inexpensive methodological aid for enrichment of plasma EVs that could help in future biomarker discoveries.

SESSION 5B

DRUGGING THE UNDRUGGABLE: TOWARDS THE DEVELOPMENT OF SELECTIVE MODULATORS OF CADHERIN-MEDIATED CELL-CELL ADHESION

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Cadherins are transmembrane calcium-dependent cell adhesion proteins that mediate cellular adherens junction formation and tissue morphogenesis. Loss of cadherin-mediated adhesion has been implicated in many different steps of tumor progression such as invasion and migration, and is strongly related to cell–cell detachment and metastasis. Altered expression profiles of epithelial E-cadherin (CDH1) and neuronal N-cadherin (CDH2) have often been observed in cancer cells, most notably in the context of the epithelial-to-mesenchymal transition (EMT) process that occurs during cancer progression. Some epithelial ovarian cancer (EOC) cells are characterized by high expression levels of E-cadherin, which facilitates EOC cell proliferation. So far, structural and mutational studies have provided a rather detailed picture of the highly dynamic cadherin homo-dimerization mechanism. However, because of this intrinsic dynamic behavior, the rational design of small ligands targeting cadherin homophilic interactions has proved difficult. We determined the crystal structure of an E-cadherin extracellular fragment in complex with a peptidomimetic compound (FR159) that partially inhibits cadherin homophilic adhesion (Fig. 1).[1]

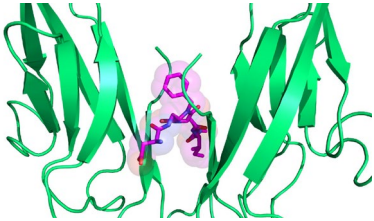

Fig. 1. Crystal structure of human E-cadherin in complex with the peptidomimetic compound FR159.

The structure, which is the first and to date the only crystal structure of an E-cadherin extracellular portion in complex with a small molecule inhibitor, reveals an unexpected binding mode and allows the identification of a druggable cadherin interface. Based on this crystal structure, we were able to identify several small molecule inhibitors that specifically modulate E-cadherin-mediated cell-cell adhesion and are effective at micromolar concentrations.[2]

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STRUCTURAL, FUNCTIONAL AND REGULATORY ASPECTS OF LEUKOTRIENE BIOSYNTHETIC ENZYMES

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Leukotrienes (LTs) are bioactive lipid mediators which play important role in different physiological and pathological processes, such as chronic inflammation, allergy, and asthma[1]. LTs are divided into two classes: a dihydroxy fatty acid, leukotriene B_4 (LTB_4), and lipid-glutathione conjugates, collectively called cysteinyl-leukotrienes (Cys-LTs). LTB_4 is a potent chemotactic biomolecule and an activator of leukocytes, while Cys-LTs induce contractions of smooth muscle in the respiratory tract. LTs are produced from arachidonic acid (AA) which is released from membrane lipids by cytosolic phospholipase A2 ($cPLA_2$). Free AA is presented to 5-lipoxygenase (5-LO) by 5-lipoxygenase-activating enzyme

(FLAP) to convert AA to 5S-hydroxy-eicosahexaenoic acid and LTA_4[2]. LTA_4 is catalyzed to LTB_4 or LTC_4 by zinc-containing soluble LTA_4 hydrolase (LTA4H) or membrane-associated LTC4 synthase (LTC_4S), respectively. LTs perform their bioactions through interactions with corresponding G protein-coupled receptors in auto- and paracrine manner. The group of enzymes in the LT pathway are co-localized at the nuclear or ER membrane upon different stimuli and form the LT biosynthetic assembly (Fig.)[1,2]. As LTs possess pro-inflammatory and immune-modulating properties, the LT biosynthetic complex is a clinically relevant target. Currently, we lack new and effective drug candidates in pre-clinical trials, therefore, there is a need for novel approaches to reduce chronic inflammation and promote pro-resolving and anti-inflammatory mechanisms. Recently, several new inhibitors have developed by our group to selectively inhibit LTA_4H (unpublished). In addition, we have demonstrated that the LT pathway can be modulated through non-canonical pathways, such as the Unfolded Protein Response in ER stress[3]. Additional structural and pharmacological studies will be carried out in the future to investigate regulatory aspects of the LT pathway.

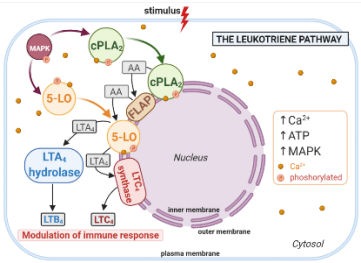

Figure. The leukotriene pathway in stimulated leukocytes.

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INTERACTION BETWEEN SIGMA-1 AND GABA-B RECEPTORS: EVIDENCE FROM SIGMA-1 RECEPTOR KNOCKOUT MICE

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The growing body of evidence suggests a significant involvement of the sigma-1 receptor (Sig1R) in the modulation of seizures[1]. FDA approval of an orphan drug fenfluramine, a mixed serotonin receptor and Sig1R ligand, for the treatment of rare epileptic encephalopathies has gained increasing interest of Sig1R as a valid target for the treatment of seizures[2]. However, the direct molecular mechanism by which Sig1R modulates seizures and the balance between the excitatory and inhibitory pathways has not been fully elucidated.

Using adult CD-1 background Sig1R knockout mice (provided by Laboratorios Dr Esteve, S.A.) our aim was to evaluate how genetic inactivation of Sig1R affects susceptibility to seizures and expression of both glutamate and GABA transporters and receptors in the brain. Sig1R knockout animals demonstrated significantly decreased tonic seizure thresholds by 28 % and 22 % in intravenous pentylenetetrazol and (+)-bicuculline infusion-induced acute seizure models, respectively. Quantitative PCR and Western blotting revealed no difference in expression of both membrane and vesicular glutamate and GABA transporters in the cortex, hippocampus and midbrain between wild-type and knockout mice. There were also no differences in the mRNA levels of the subunits of NMDA and GABA-A receptors in Sig1R knockout animals. Detailed immunohistochemical analysis showed a significantly reduced expression of the R2 subunit of the GABA-B receptors in the habenula of Sig1R knockout mice. Compared with wild-type mice, the staining intensity of GABA-B R2 in the ventral part of the medial habenula was decreased by 70 %.

Our results demonstrate significant evidence of *in vivo* interaction between Sig1R and GABA-B receptors and indicate that disturbed function of habenula might be involved in the pathogenesis of seizures.

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METHODS FOR MEASURING LIGAND BINDING PROPERTIES TO NEUROPEPTIDE Y Y1 RECEPTORS WITH NOVEL HIGH-AFFINITY FLUORESCENT LIGANDS

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In humans, the neuropeptide Y (NPY) receptor family comprises four subtypes (Y1, Y2, Y4, and Y5), which are all members of the superfamily of G-protein-coupled receptors (GPCRs). Among them, the Y1 subtype is mainly expressed in the central nervous system and regulates several processes, including food intake and blood pressure.

Ligand-receptor complex formation is a crucial step in GPCR signalling, but it requires special tools and methods to be correctly measured and analysed. Our collaborators from the University of Regensburg (Germany) synthesised a novel high-affinity derivative of established Y1 ligand BIBP3226 and labelled this with different fluorescent dyes[1]. The TAMRA labelled UR-MC026 and CM159 worked well in fluorescence anisotropy-based (FA) assay with NPY Y1-containing budded baculoviruses, and obtained binding parameters were in good agreement with results obtained with flow cytometry or with radioactive ligands. Fast and reversible binding of UR-MC026 and CM159 makes them a suitable tool for screening NPY Y1 receptor-specific ligands.

High-affinity ligand allows the determination of small receptor quantities and unlabelled ligands with similarly high affinity. However, with the development of ligands with higher and higher affinities, there comes a need for more sensitive measurement systems. We have developed a novel TIRF microscopy-based method that utilises budded baculovirus particles displaying NPY Y1 receptors immobilised to functionalised

coverslips[2]. This method allows measuring at a single-molecule level, making it suitable for measuring fluorescent and unlabelled ligands with very high affinity. High-affinity ligands can be applied to detect small amounts of receptors, for example, in tissues. However, for that purpose, Cy5 labelled ligand CM139 should be used to minimise the autofluorescence of tissue.

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SESSION 6A

STRUCTURE AND APPLICATIONS OF ssRNA BACTERIOPHAGE AP205

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The bacteriophages with single-stranded RNA (ssRNA) genomes are among the simplest and smallest of the known viruses. Structurally, ssRNA phage particles consist of a genomic RNA molecule enclosed by 178 copies of the coat protein (CP) and a single copy of the maturation protein (MP). The MP is responsible for adsorbing the virion to the cellular receptor – a pilus on the surface of the host bacterium – and subsequent delivery of the RNA genome into the cell. We have determined the cryo-EM structure of Acinetobacter phage AP205 which has revealed significant differences compared to the previously determined structures of Escherichia ssRNA phages MS2 and Qβ. Notably, the AP205 virion contains not one, but two copies of the MP assembled in an asymmetric dimer. Furthermore, the genomic RNA inside the AP205 particle is arranged in a very different manner: while in MS2 and Qβ the majority of genome-capsid interactions are formed by RNA hairpin loops, in AP205 RNA-CP interactions are mediated by double-stranded RNA segments laying tangentially with respect to the inner surface of the virion. The AP205 structure demonstrates the considerable structural flexibility for receptor recognition and genome packaging among the evolutionary highly diverse ssRNA phages.

ssRNA phages have numerous applications, one of the most significant being application of their virus-like particles (VLPs) in vaccine construction. VLPs can be used as highly immunogenic and repetitive scaffolds for presentation of various antigens. Due to externally exposed N- and C- termini of coat protein subunits, phage AP205 VLPs are particularly suitable for display of genetically fused antigens. Recently we have demonstrated usefulness of AP205 VLP platform in creating vaccine candidates against neurodegenerative diseases, such as Parkinsonism and Alzheimer's disease. Exposure of short peptide from α-synuclein on AP205 VLPs led to recognition by oligomer specific antibodies. It was also possible to produce AP205 fusions with N-terminal pyroglutamate containing Aβ

peptides, which are thought to be critical targets for Aβ plaque removing antibodies.

In summary, AP205 is a structurally unique representative of ssRNA phages and some of its properties are beneficial in construction of VLP vaccines.

VARIETY OF GRM2 TYPE BACTERIAL MICROCOMPARTMENT PARTICLES DEMONSTRATED BY CRYO-EM

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Bacterial microcompartments (BMCs) are bacterial organelles involved in enzymatic processes, such as carbon fixation, choline, ethanolamine and propanediol degradation, and others. Formed of a semi-permeable protein shell and an enzymatic core, they can enhance enzyme performance and protect the cell from harmful intermediates. With the ability to encapsulate non-native enzymes, BMCs show high potential for applied use. For this goal, a detailed look into shell form variability is significant to predict shell adaptability. We have calculated and identified four novel 3D cryo-EM maps of recombinant *Klebsiella pneumoniae* GRM2 BMC shell particles with the resolution in range of 9 to 22 Å and nine novel 2D classes corresponding to discrete BMC shell forms. These structures reveal icosahedral, elongated, oblate, multi-layered and polyhedral traits of BMCs, indicating considerable variation in size and form as well as adaptability during shell formation processes.

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SMALL ALARMONE (p)ppGpp HYDROLASE SpoT COUNTERACTS THE SYNTHETASE ACTIVITY OF RelA

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RelA-SpoT Homolog (RSH) family proteins regulate the stringent response of bacteria by controlling the levels of the signaling nucleotide alarmones such as (p)ppGpp. Ancestral RSH Rel is a bifunctional protein capable of synthesizing and hydrolyzing small alarmones. The RelA-SpoT pair arose as a result of gene duplication of Rel in Gammaproteobacteria. RelA has lost its capability of (p)ppGpp hydrolysis and evolved into a monofunctional alarmone synthesizing RSH family enzyme. In Moraxellaceae lineage of Proteobacteria just the opposite has happened with SpoT, it has lost its synthetase activity and evolved to hydrolyse small nucleotide alarmones.

In a bacterial cell, the RelA, a ribosome-associated multidomain protein, senses the amino acid starvation and responds with small alarmone synthesis. The synthesis reaction is balanced by the SpoT hydrolase activity. RelA and SpoT both share the same conserved domains, but while RelA is recruited by the starved ribosomes, it adopts highly elongated conformation. In ribosome-RelA complexes the C-terminal domains are highly structured, while the N-terminal catalytic region remains dynamic.

We have solved the crystal structure of SpoT from Acinetobacter baumannii with its native substrate ppGpp bound to the N-terminal hydrolysis (HD) domain. It reveals a compact monomeric conformation in which all the regulatory domains are wrapped around a core subdomain. The core domain in RelA is intrinsically disordered when it is in the active synthetase state, while in SpoT the core mediates allosteric crosstalk between the HD and the rest of the domains of the enzyme for its functionality. We believe that the inactive N-terminal SYNTH domain and other C-terminal domains of SpoT retain some function in stabilization and allosteric regulation of the HD domain.

POXVIRUSES AND PARAMYXOVIRUSES USE A CONSERVED MECHANISM OF STAT1 ANTAGONISM TO INHIBIT INTERFERON SIGNALING

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The induction of interferon (IFN)-stimulated genes by STATs is a critical host defense mechanism against virus infection. In this work, we show that a highly expressed poxvirus protein, 018, inhibits IFN-induced signalling by binding to the SH2 domain of STAT1, thereby preventing the association of STAT1 with an activated IFN receptor. Despite encoding other inhibitors of IFN-induced signalling, a poxvirus mutant lacking 018 was attenuated in mice. The 2.0 Å crystal structure of the 018:STAT1 complex reveals a phosphotyrosine-independent mode of 018 binding to the SH2 domain of STAT1. Moreover, the STAT1-binding motif of 018 shows similarity to the STAT1-binding proteins from Nipah virus, which, similarly to 018, block the association of STAT1 with an IFN receptor. Overall, these results uncover a conserved mechanism of STAT1 antagonism that is employed independently by evolutionarily distinct virus families.

STRUCTURAL STUDIES OF CHROMATIN PROTEINS: THE IMPACT OF CRYO-EM

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In eukaryotic cells, DNA is spooled around histone proteins forming packed units called nucleosomes, which in turn gather into higher order structures of chromatin. This scaffolding directly affects the expression of the encapsulated genes. The structural state of chromatin is determined by specific post-translational modifications to histone proteins like methylation, acetylation, and ubiquitination. These are performed by the many histone modification enzymes to either activate or silence genes. Silenced genes can condense into tightly packed chromatin occluding DNA from transcription machinery and regulatory proteins. To activate such genes, special transcription factors – pioneer factors – are capable of invading nucleosomes to facilitate recruitment of other chromatin factors and enzymes. As such, pioneer factors play the key role in cell-fate events like differentiation and cell reprogramming. We have contributed to the field with several structures of chromatin protein/nucleosome complexes together with functional studies to elucidate the mechanisms of these pivotal processes[1,2].

In the recent years, cryo-electron microscopy has developed rapidly thanks to advances in detector technology, data processing algorithms, and utilization of high-performance GPUs. The so called cryo-EM „resolution revolution" has had a tremendous impact on structural studies of chromatin proteins. Unlike crystallography, which demands rigid well-defined assemblies, cryo-EM enables the analysis of dynamic and complex mixtures. This has led to an explosion in structural data of chromatin protein/nucleosome complexes including histone modification enzymes, chromatin remodelers, and pioneer factors.

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SESSION 6B

INTERACTIONS OF KINETIC AND STOICHIOMETRIC MODELS IN THE ESTIMATION OF FLUX DISTRIBUTION IN A METABOLIC NETWORK

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Mathematical modelling of metabolism has been used for decades in systems biology to build mechanistic understanding of metabolism and it's engineering. Combination of pathway-scale kinetic models and genome-scale constraint-based stoichiometric models improve the accuracy of simulations and optimisation by the reduction of feasible solution space.

In kinetic modelling the complexity of reaction kinetics is taken into account, while it is optimistically assumed that the necessary energy, redox cofactor and some other metabolites are supplied by the remaining metabolism in some way. In the constraint based stoichiometric modelling the balance of atoms in incoming and outgoing metabolites is accounted optimistically assuming that there will be enough enzymatic power to drive the molecules through reactions.

We applied both modelling approaches to the docosahexaenoic acid (DHA) production by *Crypthecodinium cohnii*, a marine heterotrophic dinoflagellate, analysing glucose, ethanol and glycerol as substrates. Both pathway scale kinetic model and genome-scale constraint-based stoichiometric models were applied to predict biomass producing metabolic flux distributions for glucose, ethanol and glycerol based on transcriptomics[1] and ^{13}C metabolic flux analysis[2] data of DHA production by *Crypthecodinium cohnii* from glucose.

Model-based simulations and optimisations led to the reduction of optimal flux predictions done by kinetic model to enable the observed biomass production simulated by constraint-based stoichiometric model[3].

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GROWTH CHARACTERIZATION AND SUBSTRATE CONSUMPTION PATTERNS OF NON-CONVENTIONAL YEASTS IN C5-ENRICHED HYDROLYSATES FROM BIRCH

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Increased greenhouse gas emissions and strong dependencies on fossil fuels have resulted in environmental problems, such as climate change and loss of biodiversity. To change the current situation, a biobased economy offers an alternative to promote sustainable development. Biomass from lignocellulose is one of the most abundantly available resources. We investigated opportunities to develop a platform for the valorization of xylose in industrial lignocellulose hydrolysates using non-conventional microorganism. For this, nine promising yeast strains were selected from the literature and screened initially in 96-well plates on a diluted hemicellulosic hydrolysate from Birch. *Kluyveromyces marxianus* CBS 6556, *Scheffersomyces stipitis* CBS 5773, *Lipomyces starkeyi* DSM 70295, and *Rhodotorula toruloides* CCT 7815 were selected for further characterization in bioreactors, where environmental conditions were controlled. Their growth, substrate consumption patterns, and bioproducts were analyzed. *K. marxianus* CBS 6556 performed poorly, while *S. stipitis* CBS 5773 showed specific growth and sugar co-consumption rates among the highest. *R. toruloides* CCT 7815 performed the best of all four studied strains and tested conditions, showing the highest specific growth (0.23 h^{-1}) and xylose consumption (0.22 g/gdw*h) rates. This strain was able to produce 10.95 ± 1.37 gL^{-1} and 1.72 ± 0.04 mgL^{-1} of lipids and carotenoids,

respectively, under non-optimized cultivation conditions. This study provides detailed information on substrate consumption patterns that can help in future metabolic engineering or adaptive evolution experiments.

METABOLIC NETWORK ANALYSIS OF *RHODOTORULA TORULOIDES* USING STOICHIOMETRIC MODELS AND PROTEOMICS ANALYSIS

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Rhodotorula toruloides is a non-conventional, oleaginous yeast able to accumulate lipid up to 70% of its dry cell weight. It can consume wide variety of carbon substrates, including from lignocellulosic origin. To better understand its unique metabolic properties, we performed batch cultivation experiments of *R. toruloides* in mineral medium with excess xylose, glucose or acetate as a sole carbon source in nitrogen limitation and performed absolute proteome quantification during exponential growth and lipid accumulation phases. Collected physiological and protein abundance data was then used to constrain genome-scale model, rhtoGEM, with enzymatic abundances and intracellular metabolic fluxes were calculated.

Efficient biosynthesis of lipid precursors acetyl-coenzyme A and redox cofactor NADPH is important to reach high cellular lipid content. In our experiments, *R. toruloides* reached lipid content of 0.48 gram per gram of dry cell weight when grown on glucose, while the lipid yield was ca 10 and 20% lower on xylose and acetate, respectively. Metabolic flux and proteome analysis suggested different central metabolic pathways used for generating acetyl-coenzyme A and NADPH under nitrogen limitation in *R. toruloides*.

Our results demonstrated that proteome constraints in general have minor influence on intracellular flux distribution. To improve future systems biology research on *R. toruloides*, the importance of enzyme kinetics, cofactor specificity, especially of highly abundant enzymes, and incomplete gene-protein annotation should be noted. The understanding of the functions of highly abundant, but uncharacterised proteins detected in our analysis might help in the future studies of lipid production in *R. toruloides*.

DEVELOPMENT OF 3D PRINTED ENGINEERED LIVING MATERIALS FOR BIOCHEMICALS PRODUCTION

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The production of biochemicals encounter efficiency bottlenecks and lack on-demand manufacturing, which limit the advance of a sustainable circular bioeconomy at the present time. Advancements in 3D printing technologies enables the development of engineered living materials (ELMs) with novel bio-catalysis properties and on-demand deployment. 3D printing is a technology developed in the last century that allows the manufacturing of solid objects through a digitally modelled layer-by-layer deposition of materials1. Living microbial cells, such as yeast, bacteria, and algae were added to bioinks and printed with functional materials with precise 3D models recently[2]. ELMs have been employed in the areas of biosensors, encapsulation, bioenergy production, drug-releasing surfaces, and bio-catalysis[3]. Hydrogels are commonly used materials for the fabrication of composite ELMs due to their hydrophilicity, chemical stability, elasticity, biocompatibility, and tailorable mechanical properties[1,4]. In this work, hydrogel-based ELMs containing cells are going to be deployed to produce desired biochemicals. Various concentrations and materials are going to be tested for cell physiology parameters, production of biochemicals, conductivity, gas diffusion rates, and rheology. Microscopy of the fabricated structures are going to be performed for further characterizations. As a result, it is expected that the developed ELMs would have a reproducible structure with specific properties, such as cell-retaining capability, conductivity, degradability, and processability, which would

allow the enhancement of aerobic and anaerobic bio-catalysis of a portable, reusable, and on-demand technology.

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SELF-REGULATION OF THE TYPE III CRISPR-CAS SIGNALING PATHWAY

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Many prokaryotes rely on CRISPR-Cas systems for adaptive and sequence-specific protection against invading nucleic acids. In most CRISPR-Cas systems, the effector complex simply cleaves recognized nucleic acid. However, type III CRISPR-Cas systems have integrated an oligoadenylate (cA_n) signaling pathway into their mechanism of action to activate ancillary proteins. Upon foreign RNA binding, the type III CRISPR-Cas effector complex starts to synthesize cAns, which are subsequently bound by the CARF domain of accessory proteins such as HEPN ribonucleases Csm6/Csx1[1,2]. The signaling is controlled at the cAns synthesis stage by RNA transcript cleavage, which leads to the inactivation of cyclase[1,3]. For the following cAns degradation, distinct CARF ring nucleases are required[4]. However, not all organisms encoding active type III systems encode dedicated ring nucleases. By investigating, the type III-A system from *Streptococcus thermophilus* (St), we aimed to understand the regulation of the cA_n signaling pathway lacking separate ring nucleases. By tracking intracellular levels of cA_ns in a heterologous host, we identified that cells expressing the ancillary protein StCsm6 exhibit dramatically lower levels of cA_ns. cA_ns cleavage assays in vitro revealed that the CARF and HEPN domains of StCsm6 are involved in the degradation of cA_n. The CARF domain acts as a specific ring nuclease and cleaves its activator molecule cA_6, whereas the HEPN domain is responsible for the degradation of

various cA_ns – by-products of the cyclase reaction. These findings elucidate the self-regulation of the signaling pathway in type III CRISPR-Cas systems.

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INSIGHTS INTO BACTERIAL DYE-DECOLORIZING PEROXIDASES AND THEIR LIGNIN REMODELLING ACTIVITY

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Lignin, the nature's major source of aromatic chemistry, is by many seen as a green entry point alternative to fossil-based sources for chemical industry. The utilization of lignin is challenging, wherein enzymes might be the key to overcome this challenge. Lignin is a highly complex and recalcitrant substrate for enzymes as its composition depends on its source as well as on the extraction method. Therefore, the investigation of lignin-modifying enzymes was carried out concurrently with the characterization of organosolv lignins from aspen and Miscanthus x giganteus. Here we present the results of biochemical characterization of bacterial lignin-modifying enzymes called dye-decolorizing peroxidases and show that these enzymes can use organosolv lignin as a substrate.

SESSION 7

TOXIC WHEN BEING OUTSIDE: HOW EXTRACELLULAR TAU PROTEINS KILL NEURONS

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Protein aggregation is considered as hallmark for various neurodegenerative diseases including Alzheimer's, Parkinson's diseases, frontotemporal dementia, etc. Extracellular beta amyloid or intraneuronal tau aggregates are being widely investigated in relation to their neurotoxic effects. Interestingly, recent evidence suggests that intact neurons can secrete exogenous tau protein which in turn can induce fibrillization of intracellular tau in a prion-like manner, however, precise molecular mechanisms of tau pathology are not well elucidated. We have shown that extracellular tau^{2N4R} isoform, independently of its aggregation, induces phagocytosis of viable neurons by microglia activated via Toll-like 4 receptor-mediated caspase-1 pathway. Another isoform – tau^{1N4R} in oligomeric form directly caused neuronal death whereas monomeric form was not toxic. Among various tau peptides investigated, phosphorylated tau – p-tau^{2N4R} was found to be the most toxic: it induced loss of viable neurons at low nanomolar concentration and caused neuronal death at higher submicromolar concentrations. Underlying molecular and cellular mechanisms of tau neurotoxicity will be discussed in the lecture.

THE ROLE OF ENVIRONMENT IN AMYLOID AGGREGATION

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The ability to form amyloid structures may be a generic property of polypeptides, and there are two major factors which define the probability of amyloid fibril formation – amino acid sequence of the protein/peptide

and the environmental conditions. In the case of folded proteins, at least partial unfolding is necessary to trigger the amyloid formation pathway, so increased temperature, extreme pH conditions, addition of denaturants or any other changes in the environment leading to destabilization of protein structure are used in amyloid aggregation studies. Even in the case of disordered proteins, neutralization of charges or contact with hydrophobic surfaces may be necessary to induce amyloid formation. In addition to the specific conditions required for amyloid formation, changes in the environment may alter the mechanism of aggregation and lead to distinct amyloid fibril conformations. Finally, environment conditions affect the kinetics of aggregation and may alter the effect of anti-amyloid compounds.

I'd like to present our recent findings of our group related to environment-dependent polymorphism of amyloid fibrils [1-5] and to the role of environment in detection of anti-amyloid compounds [6-7].

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COPPER METABOLISM IN ALZHEIMER'S DISEASE

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Copper ions play a crucial role in cellular energy production, antioxidative defense and oxidative metabolism. However, free copper ions are toxic and copper metabolism is therefore highly regulated. Dysregulation of

copper homeostasis occurs in multiple diseases, including Alzheimer's disease (AD). According to the prevailing opinion, there is an excess of copper in extracellular and copper deficit in intracellular regions of AD brain. Therefore regulation of copper homeostasis in AD needs a delicate approach, which takes into account copper metabolism in intracellular as well as extracellular environments. Earlier we have determined Cu(I)-binding affinities of major intracellular Cu(I) proteins as well as Cu(II)-binding affinity of amyloid-beta (Aβ) peptides. In parallel, we have also determined Cu(I)-binding properties of a series of copper chelators, including WD drugs. Taking all data together we obtained a comprehensive understanding of human copper metabolism and molecular tools for its regulation. Most promising copper-binding ligands, were tested in cellular and insect AD models. We demonstrate that the natural intracellular copper chelator, α-lipoic acid (LA) translocates copper from extracellular to intracellular space in an SH-SY5Y-based neuronal cell model and is thus suitable to alleviate the intracellular copper deficit characteristic of AD neurons. Furthermore, we show that supplementation with LA protects the Drosophila melanogaster models of AD from developing AD phenotype by improving locomotor activity of fruit fly with overexpression of human Aβ with Iowa mutation in the fly brain. In addition, LA slightly weakens copper-induced smooth eye phenotype when amyloid-β protein precursor (APP) and beta-site APP cleaving enzyme 1 (BACE1) are overexpressed in eye photoreceptor cells (1).

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PATHALOGICAL AND FUNCTIONAL AMYLOIDS SEEN BY SOLID-STATE NMR SPECTROSCOPY

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The solid-state NMR (ssNMR) spectroscopy) is a powerful toll to study proteins at atomic level close to their native state. Here we are going to demonstrate ssNMR applications to study proteins, which are forming

neuropathological disease associated amyloid fibrils like - tau, abeta and alpha-synuclein. We will also show a new efficient technique to biochemically incorporation fluorine labelled amino acids for ssNMR studies into functional amyloid model systems in order to expend further applications of ssNMR. In the presentation we will also demonstrate the combination of our ssNMR data with other biophysical methods obtained for amyloid fibrils.

CELL-PENETRATING PEPTIDES WITH UNEXPECTED ANTI-AMYLOID PROPERTIES

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Cell-penetrating peptides (CPPs) with sequences derived originally from a prion protein (PrP) have been shown to exhibit both anti-prion and anti-amyloid properties particularly against prion proteins and the amyloid-β (Aβ) peptide active in Alzheimer's disease. These disease-modifying properties are so far observed in cell cultures and in vitro. The CPP sequences are composed of a hydrophobic signal sequence followed by a highly positively charged hexapeptide segment. The original signal sequence of the prion protein can be changed to the signal sequence of the NCAM1 protein without losing the anti-prion activity. Although the detailed molecular mechanisms of these CPP peptides are not fully understood, they do form amyloid aggregates by themselves, and molecular interactions between the CPPs and PrP/Aβ can be observed in vitro using various spectroscopic techniques. These initial intermolecular interactions appear to re-direct the aggregation pathways for prion/amyloid formation to less cell-toxic molecular structures (i.e., co-aggregates), which likely is why the disease-inducing PrP/Aβ aggregation is counteracted *in vivo*.

SESSION 8

MODULATION OF STEVIA SECONDARY METABOLISM WITH COLD PLASMA

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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, sweeteners and non-sweetener fraction of stevia plants are intensively investigated.

Seed treatment with non-thermal or cold plasma (CP) 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. 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. This stimulating effect 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 had 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.

PURINE AUXOTROPHIC STARVATION EVOKES QUESCIENCE LIKE PHENOTYPE IN THE BUDDING YEAST

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Purine synthesis pathway is well conserved among eukaryotes. Interestingly, purine auxotrophy is a typical trait of intracellular parasites, like toxoplasma and leishmania. Also many budding yeast *Saccharomyces cerevisiae* laboratory strains are purine auxotrophs.[1] We use budding yeast as a model to explore phenotypic features that are brought when transition from purine phototroph to purine auxotrophy occurs. Specifically, we explored purine starvation impact on metabolism, stress resistance and the global gene expression level.

Budding yeast effectively stops metabolism and initiates quiescence phenotype in the case of depletion of carbon or nitrogen sources. We wondered if anything happens in the case of purine starvation. Indeed, we observed that purine starved cells stop their cycle in G1/G0 state, become tolerant to severe environmental stresses. Also intracellular RNA concentration decreases and massive downregulation of ribosomal biosynthesis genes occurs.[2] In the same time, purine starvation phenotype develops via expression of specific genes, distinct from non-starving. Rim15p and it's downstream effectors Msn2/4p upregulate genes responsible for stress resistance phenotype. Yet, how purine depletion is signalled to Rim15p is not known.

We think that purine auxotrophic starvation induces phenotype which in many aspects' mimics „natural" nitrogen or carbon starvations, thus resembling stationary phase or quiescent cells. Our results demonstrate that organized metabolic response is initiated not only via „natural starvations", but also when starving for metabolic intermediates, like purines. Moreover, our findings are in line with observations in other

eukaryotic purine auxotrophs, which in the case of purine starvation arrest their cell cycle and increase resilience against many environmental stresses.

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LONG-CHAIN ACYLCARNITINES: FROM MITOCHONDRIAL METABOLISM TO CLINICAL APPLICATIONS

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Mitochondria play a critical role in the generation of metabolic energy in eukaryotic cells, and disturbances in mitochondrial function can cause a vast array of diseases. Mitochondrial disorders that are characterized by incomplete fatty acid oxidation due to lack of some enzyme or transporter activity induce accumulation of long-chain acylcarnitines in mitochondria. Meanwhile, in cardiac mitochondria, long-chain acylcarnitines inhibit pyruvate and lactate metabolism even at physiological concentrations measured in the fasted state. At elevated levels, the accumulation of long-chain acylcarnitines in mitochondria inhibits oxidative phosphorylation, induces mitochondrial membrane hyperpolarization, and stimulates ROS production. Thus, the high mitochondrial content of long-chain acylcarnitines is expected to increase the risk of mitochondrial and cardiac damage, particularly in conditions of cardiac ischemia.

Inhibition of long-chain acylcarnitine synthesis has been confirmed as a promising treatment approach in various disease models, including myocardial infarction, atherosclerosis, and insulin resistance. In recent studies, a pharmacological decrease in long-chain acylcarnitines has been studied in models of cardiometabolic diseases, inherited disorders, cancer,

endotoxemia- and pulmonary hypertrophy-induced cardiac damage, thus opening new horizons for long-chain acylcarnitine-lowering and mitochondria-protective strategies.

Plasma acylcarnitine measurements are widely used for diagnosis and characterization of inherited fatty acid oxidation disorders. Recently, we started the development of a novel diagnostic method that characterizes cardiac tissue-specific insulin resistance. We tested whether a decrease in circulating long-chain acylcarnitine concentrations after glucose administration in a glucose tolerance test is associated with insulin sensitivity and can be used for the diagnosis of insulin resistance. We found that the measurements of changes in plasma long-chain acylcarnitine concentrations after glucose load in fasted subjects are useful as diagnostic markers for heart and muscle-specific insulin resistance in clinics.

In summary, long-chain acylcarnitines are important mitochondrial energy metabolism actors that pave new possibilities for biomarker and therapeutic target discovery.

DEVELOPMENT OF COVALENT BISUBSTRATE INHIBITOR OF CYCLIC AMP DEPENDENT PROTEIN KINASE

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New possibilities have emerged for the development of protein kinase (PK)-targeted drugs with covalent inhibition that takes advantage of the reactive cysteine residues in and around the ATP-binding site[1,2]. However, more than 300 human PKs lack a cysteine residue in the ATP-binding site, which complicates targeting these PKs with covalent inhibitors[3-5]. We developed a series of covalent bisubstrate PK inhibitors equipped with an electrophilic warhead, which reaches out of the ATP-binding site and reacts with a cysteine residue at the substrate binding site of the catalytic subunit of cAMP-dependent protein kinase (PKAc). The most potent inhibitors featured high affinity (K_D = 4.9 ± 0.1 pM for ARC-2165) and high k_{inact}/K_I ratio (6.2 × 10^7 M^{-1}/s^{-1} for ARC-2166) for the reaction with PKAc. Fluorescent dye-labelled covalent bisubstrate inhibitors demonstrated high PKA-selectivity in the cell lysates under optimized conditions and

were able to penetrate the cellular membrane and react with targets in live cells. The bisubstrate covalent inhibitors could serve as lead compounds for targeting other PKs that possess an analogously positioned cysteine residue.

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ALBUMIN AFFECTS THE STABILITY, OLIGOMERIZATION AND LIGAND INTERACTIONS OF LIPOPROTEIN LIPASE

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Lipoprotein lipase (LPL) is a crucial enzyme in lipoprotein metabolism and a potential drug target for the treatment of hypertriglyceridemia. LPL can appear in different oligomeric states and its activity is influenced by a complex ligand network. In the current study we demonstrate that a major component of blood plasma, albumin, forms a complex with LPL and influences its oligomerization, stability and ligand interactions. The LPL-albumin complex was strong enough to appear in all physiological locations of LPL. We reveal an interplay between albumin and heparin that could provide an additional mechanism for ensuring the dissociation of heparan sulfate proteoglycan-bound LPL oligomers into active LPL upon secretion. Additionally, great consideration into LPL concentration and buffer environment should be taken in studies to distinguish between irreversible inactivation or aggregation and reversible LPL oligomer formation, which might affect interactions with various ligands and drugs.

POSTER SESSION

1. INVESTIGATING THE EARLY STAGES OF MISFOLDING OF AMYLOID-FORMING PEPTIDE PAP248-286 USING MOLECULAR DYNAMICS SIMULATIONS

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HIV has infected more than 75 million people since its inception and, so far, has claimed the lives of more than 36 million people worldwide. The one very crucial natural factor that has been identified to play a key role in HIV transmission is fragments of the prostatic acidic phosphatase (PAP) protein. The PAP248-286 peptides aggregate and form amyloid fibrils termed Semen-derived enhancers of viral infection (SEVI) that capture HIV particles and strongly enhance the number of productively infected cells by promoting virion-cell attachment and fusion. To investigate the early stages of misfolding of PAP2482-86 peptide and to see the effect of different pH environments we have employed molecular dynamics simulations. Our data showed that turn formation in PAP248-286 peptides is the first step in the multi-stage misfolding process, and our study also showed the econdary structure of PAP248-286 is sensitive to the pH environment. Overall, the study elucidates misfolding of PAP248-286 at the atomic level and opens new avenues in structure-based drug design against PAP248-286 peptides[1].

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2. METAGENOMIC ANALYSIS OF BACTERIAL COMMUNITIES OF THE EDIBLE INSECT THE HOUSE CRICKET

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Background: Novel foods represents nutritional, economic, and ecological benefits. It is alternative to conventional food and traditional farming. The insect usage in the food industry is increasing worldwide due to high nutritional values, environmentally friendly cultivation, and effective feed conversion ratio during mass rearing. The house cricket (*Acheta domesticus*) is considered as one of the most promising specie for insect-based food production. Regarding that, crickets must comply food safety requirement.

Objective: The aim of this study was to describe bacterial communities inhabiting the surface and whole-body of house cricket *Acheta domesticus* and evaluate their microbiological safety.

Methods: The amplicon-based next-generation sequencing (NGS) and bioinformatics analysis.

Results: Based on sequencing of the 16S rRNA domain data, bacterial communities on the surface and whole-body of house crickets were determined. The most common phylum was Bacteroidetes, following by Proteobacteria, Firmicutes and Verrucomicrobia. On the genus level, the most encountered were *Parabacteroides*, *Bacteroides* and *Dysgonomonas*. Potential species of these genera are mostly inhabitants of the gastrointestinal tract of animals or humans, but some of them may can act as opportunistic pathogens. Scientific studies on the safety risks in edible crickets need to be carried out for markets and consumers, therefore the obtained knowledge could be used to formulate the most efficient raw cricket production steps and to set conditions for avoiding bacterial risk.
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3. MIRNAS AS POTENTIAL BIOMARKERS FOR LUNG CANCER

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Lung cancer is the leading cause of cancer death world-wide. There are two main types of lung cancer – small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is the most common form accounting for more than 80 % of cases while SCLC is a less common but more aggressive. The early diagnosis and control of cancer metastasis are critical factors in cancer treatment. Discovery of novel biomarkers and therapeutic targets have helped to increase survival rates for many types of cancer, including lung cancer. Over the past decade miRNAs have emerged as potential biomarkers and targets in lung cancer diagnosis and treatment. miRNAs are small 20-25 nucleotide long single-stranded non-coding RNAs that play an important role in tumor initiation and growth. miRNAs function in post-transcriptional regulation of gene expression and can act as tumor suppressors, oncogenes or metastasis regulators.

The previous study at the National Cancer Institute, Vilnius, Lithuania identified a set of miRNAs that can be associated with lung cancer metastasis[1]. A number of miRNAs were selected for further investigations into their expression signatures in normal and cancerous lung tissue biopsies with the aim to identify novel potential biomarkers for lung cancer.

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4. RYEGRASS MOTTLE VIRUS PROTEASE STRUCTURE AND PROTEOLYTIC PROPERTIES

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Sobemoviruses encode serine-like 3C proteases (Pro) which participate in polyprotein processing and protein maturation. Its activity *in trans* is mediated by naturally unfolded VPg. D-H-S forms catalytic triad; modification of its residues terminates polyprotein processing. We had demonstrated that for RGMoV Pro activity *in trans* could be achieved with free VPg co-expression without fusion. We created three RGMoV Pro variants for 3D structure determination – 2 Δ117Pro catalytically active forms – one as VPg-free form, second in VPg-bound state with fused VPg at the C-terminal part (Δ117Pro-E/A-VPg) and one catalytically defective $\Delta117Pro^{cm}$ form with S^{159}/A mutation. All variants were well expressed and soluble in *E. coli* system and can be purified easer by IMAC and/or gel filtration. After 3D structure determination the overall fold of Δ117Pro is typical for CLP. VPg residues 256-265 in the Δ117Pro-E/A-VPg forms a short α-helix that binds to the surface of Δ117Pro and W^{260} is the key residue in this interaction. VPg causes several significant conformational shifts in the Δ117Pro structure: 1) VPg absence L^{87} and eIb β-strand shifts towards the VPg binding pocket, disrupting its β-strand conformation and altering the position of the loop between eIb and fI strands; 2) the α1 helix in VPg-free structure is also slightly shifted towards the empty VPg binding site; 3) D^{92} chain A it is rotated away from the catalytic triad H^{49}, disrupting the functionality of the catalytic triad; 4) the partial unwinding of βII barrel and subsequent movement of bII and cII β-strands and cII/dII loop and the disordering of eII/fII loop rendering the Glu-binding site less suitable for cleavage site recognition. This shift also could render the Glu-binding site less suitable for cleavage site recognition. We failed to develop FRET for Pro catalytic activity *in cis* to test and evaluate VPg impact on catalytic speed. But peptide cleavage was detected by robust MS analysis. Additionally, we performed *in vitro* cleavage test using $\Delta50Pro^{cm}$ as substrate to monitor VPg possible Pro in cis cleavage speed modulation. We observed that VPg do not increase Pro cleavage speed. Ca^{2+}, Zn^{2+} inhibited, but Mg^{2+} slightly reduced Pro activity.

5. THE EFFECT OF HYPOXIA/REOXYGENATION ON RENAL CELLS MITOCHONDRIAL FUNCTION

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Kidney hypoxia/reoygenation occurs during ischemia/reperfusion, a pathological condition, which causes to mitochondrial injury [1]. The aim of this study was to evaluate the effect of hypoxia/reoxygenation on renal cell viability and mitochondrial oxidative phosphorylation system. Hypoxia in vitro was induced by sealing cell culture dishes in bags filled with nitrogen and 2 % oxygen gas mixture at 37 °C, for reoxygenation cell dishes were removed from the bags and medium was changed. Mitochondrial function was evaluated by measuring respiration using high resolution respirometry system Oxygraph-2k. Cell viability was evaluated by using fluorescence microscopy. Cell viability was not affected by hypoxia and reoxygenation, however 24-hour hypoxia and 48-hour reoxygenation increased cell apoptosis. Hypoxia and reoxygenation inhibited cell proliferation. 24-hour hypoxia decreased mitochondrial respiration in state 3, maximal mitochondrial respiration and uncoupled respiration. 24- and 48-hour reoxygenation did not restore mitochondrial respiration to control levels. However, after 24-hour hypoxia and 48-hour reoxygenation mitochondrial respiration rates in all respiration states decreased. Hypoxia and reoxygenation decreased the respiratory control index compared to control group, however it did not alter the permeability of outer or the inner mitochondrial membranes. In conclusion, hypoxia and hypoxia/reoxygenation did not affect the viability of renal proximal tubular epithelial cells, however it inhibited cell proliferation and mitochondrial function, that did not recover to control levels after reoxygenation.

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6. MERCURY ION BINDING TO APOLIPOPROTEIN E VARIANTS APOE2, APOE3, AND APOE4: SIMILAR BINDING AFFINITIES BUT DIFFERENT STRUCTURE INDUCTION EFFECTS

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Mercury intoxication typically produces more severe outcomes in people with the *APOE-ε4* gene, which codes for the ApoE4 variant of Apolipoprotein E, compared to individuals with the *APOE-ε2* and *APOE-ε3* genes. Why the *APOE-ε4* allele is a risk factor in mercury exposure remains unknown. One proposed possibility is that the ApoE protein could be involved in clearing of heavy metals, where the ApoE4 protein might perform this task worse than the ApoE2 and ApoE3 variants. We used fluorescence and circular dichroism (CD) spectroscopy to characterize *in vitro* interactions of the three different ApoE variants with Hg(I) and Hg(II) ions. Hg(I) ions displayed weak binding to all ApoE variants, and induced virtually no structural changes. Thus, Hg(I) ions appear to have no biologically relevant interactions with the ApoE protein. Hg(II) ions displayed stronger and very similar binding affinities for all three ApoE isoforms, with KD values of 8.5 ± 0.25 μM for ApoE2, 8.7 ± 3.2 μM for ApoE3, and 8.7 ± 0.64 μM for ApoE4. Binding of Hg(II) ions also induced changes in ApoE superhelicity, i.e., altered coil-coil interactions, which might modify protein function. As these structural changes were most pronounced in the ApoE4 protein, they could be related to the *APOE-ε4* gene being a risk factor in mercury toxicity.

7. ENZYME ENGINEERING OF FRUCTOSYL PEPTIDE OXIDASE TO WIDEN ITS ACTIVE SITE ACCESS TUNNEL AND IMPROVE ITS THERMAL STABILITY

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Enzyme engineering is a tailoring process that allows the modification of naturally-occurring enzymes to provide them with improved catalytic efficiency, stability or specificity. By introducing partial modifications to their sequence and to their structural features, enzyme engineering can transform natural enzymes into more efficient, specific, resistant biocatalysts and render them suitable for industrial processes.

In our lab, we focus on a class of enzymes called Fructosyl Amino Acid Oxidases (FAOX), which are flavoproteins that catalyze the oxidation of fructosyl amino acids to form glucosone, amino acid and hydrogen peroxide which has major role in the management of diabetes, and specifically in the detection of glycated hemoglobin (HbA1c).

However, naturally occurring FPOX are not able to detect HbA1c directly because these enzymes show no significant activity on intact proteins due to the buried active site and to the narrow tunnel that provides access to their catalytic pocket, depicted by the crystal structures of FAOX and FPOX enzymes. Hence, the need to expand their substrate range by enzyme engineering.

We applied a rational design approach to engineer a novel enzyme with a wider access tunnel to the catalytic site, using a combination of Rosetta design and molecular dynamics simulations.

We have been successful in designing several mutants shows a significantly wider and shorter access tunnel, relative to the wild-type (WT) enzyme. Upon experimental testing, engineered enzyme shows good structural stability and maintains significant activity relative to the WT. Also, the thermal stability of WT enzyme has been improved with the variants that have increased salt bridges, improved RMSF, improved native contacts, and disulphide bonds.

We are currently working to determine the structures by X-Ray Crystallography of the engineered enzyme, study its biophysical properties,

determine its activity and further increase its thermal stability by embedding them in a biomaterial.

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8. MOLECULAR AND STRUCTURAL BASES OF THE PLASMODIUM FALCIPARUM THREONYL-TRNA SYNTHETASE INHIBITION

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Malaria is one of the diseases that continues to be a global health problem (229 million cases of malaria in 2019) [1], especially in developing countries. The resistance that the main transmitting parasites (*Plasmodium falciparum* and *Plasmodium vivax*) have acquired to existing antimalarial drugs justifies the search for new antimalarial drugs [2]. A recent approach is to target the protein translation machinery of the parasite. In this research project, we want to explore the potential of threonyl-tRNA synthetase (ThrRS) as a possible source of antimalarial drug targets. The first step in our project is to obtain a *Plasmodium falciparum* construct (pf_ThrRS) to facilitate fragment-based drug design (FBDD) studies and subsequent co-crystallization with native and optimized compounds found during interaction experiments. One of the main problems we have to face is the fact that both proteins, the human (Hu_ThrRS) and the (pf_ThrRS) share 56% sequence identity and the amino acid residues involved in the interaction between (Hu_ThrRS) with inhibitors such as borrelidin (a natural threonyl-tRNA synthetase inhibitor) they are completely conserved in (pf_ThrRS). In this case, borrelidin stands out for its strong antimalarial effect. However, it also inhibits (Hu_ThrRS) and is very toxic to human cells [3]. Therefore, the main objective of our research is to find threonyl-tRNA synthetase inhibitors that are specific for (pf_ThrRS) and with minimal or negligible toxicity to human cells.

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9. PHOTOSWITCHABLE LIGANDS AS TOOL TO MODULATE BIOLOGICAL ACTIVITY

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In contrast to other tools for the control biological processes, light does not cause contamination of the studied object, has low or negligible toxicity at long wavelengths and can be delivered with very high spatial and temporal precision. The use of light in conjunction with a pharmacological approach gives rise to the ever-expanding field of photopharmacology. Indeed, as pharmacodynamics and pharmacokinetic properties of drugs are directly related to their molecular structure, a photoinduced structural change in a photoswitchable drug can result in the activation/deactivation or modulation of the drug-target interaction and biological functions only in the region where light is provided. To date, photopharmacology has been extensively used to manipulate biological activity at the cellular level by targeting ion channels, G protein-coupled receptors, enzymes and protein-protein interactions.[1]

One of the major advancements in photopharmacology would be to develop drugs that can be photoswitched using low energy light, which is

less scattered in tissue and can penetrate deeper in the body. To this end, a molecular structure should be designed to ensure photoconversion by red or infrared light. Here, we present the molecular design of a novel γ-aminobutyric acid type A receptors (GABAARs) ligand derivative that displays photochromic properties with red light and we discuss its chemical as well as its photoisomerization and photopharmacological properties.[2]

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10. SYNTHESIS AND ASSEMBLY OF *SACCHAROMYCES CEREVISIAE* L-BC VIRUS-LIKE PARTICLES

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Virus-like particles (VLPs) are nanosized vehicles made up of self-assembled viral proteins that mimic the structure of native virus. Due to the lack of genetic material, they are non-infectious and therefore safe to use[1]. Free of genetic material inside, VLPs can be loaded with various compounds such as oligonucleotides, peptides, proteins, magnetic nanoparticles, or drug molecules[2]. Such nanocarrier systems can be easily developed in the laboratory by expression of recombinant viral proteins and their self-assembly into VLPs.

Most strains of *Saccharomyces cerevisiae* carry one or both double-stranded RNA viruses, L-A and L-BC. Since these viruses do not have any known extracellular route of transmission, they are considered as non-infectious[3]. Also, *S. cerevisiae* is an attractive system for recombinant protein expression because of its simple cultivation, post-translational protein modification and high yield of recombinant protein[4].

The aim of this study was to develop an expression system of recombinant *S. cerevisiae* L-BC virus major capsid protein Gag, that allows the in vivo assembly of corresponding VLPs.

For the expression of L-BC virus gag gene we used S. cerevisiae cells and to concentrate and purify self-assembled VLPs from the cell lysate, both sucrose cushion and CsCl density gradient ultracentrifugation had been performed. Fractions with the biggest amount of Gag protein were pooled and analyzed by TEM. In this work, we demonstrate that recombinant L-BC Gag protein, expressed in *S. cerevisiae cells*, self-assembles into individual VLPs.

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11. SEMI-TARGETED SEQUENCING OF FUSION TRANSCRIPTS IN PROSTATE CANCER ENABLED BY OLIGONUCLEOTIDE-MODIFIED DIDEOXYNUCLEOTIDES

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Chromosomal rearrangements are the most common genetic changes in cancer genomes and they often lead to the formation of gene fusions which may be transcribed into fusion transcripts. Current detection methods rely on PCR- or hybridization-based techniques that do not allow the detection of novel fusion breakpoints. To overcome these challenges, we developed a new rapid and accurate identification method, termed

fusion sequencing via terminator-assisted synthesis (FTAS-seq), for high-throughput gene fusion profiling.

We developed efficient and straightforward synthesis strategy of oligonucleotide-tethered dideoxynucleotides (OTDDNs) to capture unknown sequences downstream of the target site. Modified OTDDNs, upon random incorporation during primer extension reaction, create DNA fragments of a desired average length, with simultaneous labeling of a corresponding DNA strand with sequencing adapter. Oligonucleotide modification then serves as a priming site for subsequent synthesis of cDNA strand. We applied FTAS-seq to study *TMPRSS2-ERG* fusion transcripts in prostate cancer cell line NCI-H660 and in clinical prostate cancer RNA samples. We identified 3 previously described chimeric transcripts in NCI-H660 RNA as well as one new possible variant. Analysis of clinical samples showed that FTAS-seq is more sensitive approach than conventional PCR-based methods: 3 TMERG fusion transcripts, which were previously detected with amplification-based methods, and 10 other possible variants were detected. It is a good alternative to amplicon sequencing as it has greater discovery potential at the same level of cost-effectiveness.

12. LIGAND AUGMENTATION IN THE ACTIVE SITE OF CARBONIC ANHYDRASE LEADS TO THE DISCOVERY OF CAIX-SELECTIVE INHIBITORS

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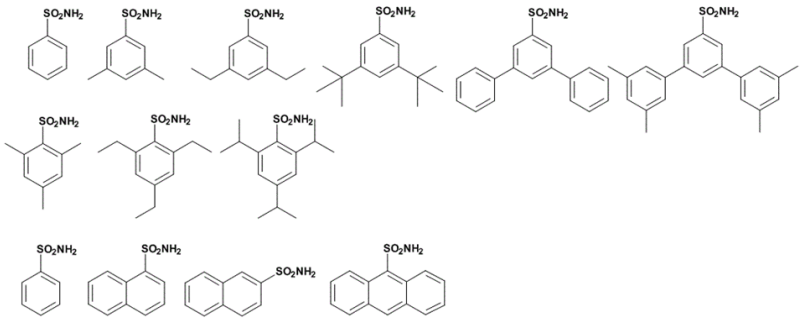
Carbonic anhydrases (CA) are ubiquitous metalloenzymes which catalyze reversible hydration of carbon dioxide to a bicarbonate anion[1]. This reaction is of fundamental importance to many physiological and pathological processes. Many carbonic anhydrase isozymes are important therapeutic targets with the potential to be inhibited to treat a wide range of disorders. Notably, CA IX is overexpressed in hypoxic cancer cells and is expressed in a very limited number of normal tissue. Therefore, CAIX

isozyme is defined as a promising drug target. A challenge in the design of CAIX-selective inhibitors is related to the high number of CA isozymes and high similarity of their active site pockets.

The major class of CA inhibitors is based on compounds bearing a primary sulfonamide group. Sulfonamide-based CA inhibitors are widely used as therapeutic agents. Benzenesulfonamides are considered the most abundant type of sulfonamide-class inhibitors. We have synthesized a series of benzensulfonamides bearing hydrophobic substituents of a systematically increasing size[2]. Variation of the hydrophobic substituent length and bulkiness was performed on the benzenesulfonamide scaffold by three approaches: substituting 3,5-positions; substituting 2,4,6-positions, and extending the condensed ring system. The gradual augmentation of the inhibitor size allowed the determination of the maximal contact area and maximal size that could fit in the protein pocket. This led to an exploration of active site cavities giving new insights into the rational design of CAIX-selective inhibitors.



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13. PROTEIN INDUCED PHOSPHORESCENCE AND ITS AMPLIFICATION THROUGHT THE ENERGY TRANSFER

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More than a decade we have developed organic probes with protein binding dependent long lifetime luminescence and applied these ARC-Lum probes for biochemical analyses of protein kinases. The cores of the probes are phosphorescent fragments that bind to the ATP pockets of kinases. The kinase scaffold protects the exited triplet state of the phosphorescent unit from the quenching by dissolved oxygen and molecular movements. This kind of protein induced phosphorescence is still weak and is applicable for assays only in the case of bright organic phosphors and sensitive instrumentation. When the phosphorescent fragment has conjugated with a suitable fluorescent dye, FRET can take place resulting in significant amplification of the long lifetime luminescence signal. Efficient FRET between triplet exited state of the donor to the singlet exited state of the acceptor fluorophore requires overlap between absorption spectrum of the fluorescent dye and phosphorescence spectrum of the phosphorescent moiety. FRET from the triplet state is a forbidden transfer, but may be still be orders of magnitude faster than forbidden transfer that correspond to phosphorescent emission. This faster rate of triplet-singlet FRET ables to compete with non-radiative decay processes and leads to bright long lifetime luminescence emission from the probe-kinase complex. Overall, these tandem luminophors are in many aspects comparable with lanthanide probes and similar time-gated instrumentation could be used.

Analyses of AGC kinases and their inhibitors[1] was the first application of these ARC-Lum probes and later this approach has been widened to other kinases like CK2[2]. Currently we are working to expand the area to other kinases and even to other classes of proteins.

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14. EPILEPTIC SEIZURE SUPPRESSION BY XENOGRAFT OF ENGINEERED HUMAN WHARTON'S JELLY MESENCHYMAL STEM CELLS IN KINDLING MODEL

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Epilepsy is a chronic neurological disorder that needs innovative molecular and cellular approaches to address unmet drug resistance epilepsy in 30% of patients. To push preclinical studies forward, we targeted the human adenosine kinase gene (*ADK*), adenosine removing key enzyme, in human Wharton's jelly mesenchymal stem cells (hWJMSCs) by a lentiviral anti-*ADK* miR-shRNA vector. In this study, we enhanced the therapeutic potential of hWJMSCs as adenosine-releasing stem cells by knockdown of *ADK*, for suppressing seizures in a kindling model of epilepsy among male Wistar rats (1). After the lentiviral transduction of hWJMSCs with anti-*ADK* miR-shRNA expression cassette, we implicated the downregulation of *ADK* up to 95% in RNA and protein level by qRT-PCR and western blot, respectively. Adenosine concentration reached 10 ng per ml of the culture medium when incubating 10^5engineered hWJMSCs for 8 hours. Cell transplantation in pentylenetetrazole-induced kindled rats significantly decreased the amplitude, duration, and seizure spike frequency while increased the latency of the appearance of the first seizure spike on days 7 and 14 of EEG recording. Behavioral seizure monitoring showed complete protection from convulsive seizures in 100% (n=20) and 83% (n=18) of kindled rats for the first and second weeks after cell graft respectively. An animal showed complete seizure protection (n=16) after 8 weeks. Our findings suggest that adenosine releasing hWJMSC might be a striking source in cell-based gene therapy and may have a therapeutic perspective in epilepsy (2).

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15. *THERMOTT*: TOOL FOR PROTEIN STABILITY AND PROTEIN-LIGAND INTERACTION ANALYSIS

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Differential scanning fluorimetry (DSF; also known as thermal shift assay, TSA) is a quick, easily accessible and inexpensive method to determine protein stability (melting temperature, T_m) at different conditions. This method is often used in drug discovery for high-throughput screening of ligand libraries.

DSF data can be used to quantitatively determine protein-ligand binding affinities (K_b). Despite that, its most common use is limited to only observing a shift in T_m, i.e., only to see whether the ligand binds at all. This is a consequence of a lack of user-friendly software that would perform the necessary regression analysis of complex thermodynamic equations.

For this reason, we developed *Thermott*[1] – a tool capable of performing such analyses from start to finish. From experimental DSF data it can determine protein melting temperatures and subsequently determine protein-ligand binding affinities (fig. 1). *Thermott* is available as a free, open-source online web application and requires no additional setup (https://thermott.com/). We believe that our tool greatly simplifies and streamlines the analysis of DSF data and thus will make this method much more accessible.

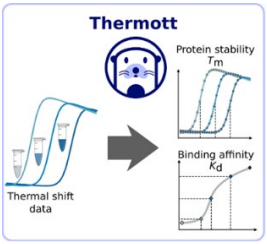

Figure 1. Thermott workflow.

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16. PRODUCTION OF MICROALGAE FOR FISH FEED USING FLUE GAS AS AN INPUT

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European Union's long-term goals on climate neutrality by 2050 target establishing a green, sustainable and healthy future to be a climate-resilient society. The immediate EU wide targets of 2030 climate and energy framework focus on reducing greenhouse gas emissions to at least 55% and utilization of biomass for energy efficient applications [1]. Algae, being a sustainable solution in regulating these emissions, is gaining widespread recognition in Europe considering its capability to regulate the CO_2 levels [2,3]. In this investigation, we compare the growth rate of different types of microalgae by exploring their CO_2 sequestering nature. To evaluate the influence of the CO_2 uptake in their growth and biomass production, flue gas was introduced to the algal bioreactors in different concentrations. The utilization of industrial flue gas for the algal growth encourages the waste management and recycling concept of circular economy. Furthermore, the microalgae with highest biomass production will be utilized as a fish feed material to improve the nutritional value, and to enhance the biosynthesis of red carotenoid pigment – astaxanthin in the salmon and rainbow trout fish thus, guaranteeing the complete valorisation of algal biomass towards the creation of value added products [4]. Moreover, the toxicity concerns and product quality are ensured by consistent toxicity evaluations of the algal biomass and fish models.

Keywords: microalgae, CO_2 sequestration, bioreactors, industrial flue gas, astaxanthin, fish feed.

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17. ACTIVITY AND FUNCTIONALITY ANALYSIS OF *STREPTOMYCES* AND *ARTHROBACTER* BACTERIAL CUTINASES

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Cutinases are esterases degrading the polyester cutin as well as other polyesters such as polyethylene terephthalate (PET) and polycaprolactones (PCL). While PCL is biodegradable, PET is categorized as a non-biodegradable polymer[1] and it has become a major component of plastic waste found in the environment, moreover, the recycling process has also been shown to have its flaws[1]. One solution is using PET degrading enzymes. The *Streptomyces scabiei* cutinase has also been shown to degrade PET[2], however, knowledge surrounding this cutinase is limited. Another cutinase facing the same knowledge gap is the *Arthrobacter* cutinase, despite the sequences encoding the cutinase being accessible in various gene databases. For these reasons, it is of highest importance to fill the gap of fundamental knowledge surrounding cutinase enzymes, which have the potential to solve plastic waste problems in eco-friendly ways.

In this study, mutants of the *S. scabiei* 87.22 cutinase (Asp94Ala, Ser123Ala) as well as the *Arthrobacter* sp. SLBN-53 (Ser85Ala, His176Ala) cutinase were created. The influence of the mutations on the catalytic activity and overall functionality of the enzymes were assessed. The mutants were created using the overlap extension method, purified using immobilized metal affinity chromatography (IMAC) and the activity as well as physiochemical characterization was carried out spectrophotometrically

using synthetic *p*-NP esters as substrates. Obtained results highlighted the importance of the selected amino acids on the functionality of target bacterial cutinases.

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18. FASTER GROWTH ENHANCES LOW CARBON FUEL AND CHEMICAL PRODUCTION THROUGH GAS FERMENTATION

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Gas fermentation offers both fossil carbon-free sustainable production of fuels and chemicals and recycling of gaseous and solid waste using gas-fermenting microbes. Bioprocess development, systems-level analysis of biocatalyst metabolism, and engineering of cell factories are advancing the widespread deployment of the commercialised technology. Acetogens are particularly attractive biocatalysts but effects of the key physiological parameter – specific growth rate (μ) – on acetogen metabolism and the gas fermentation bioprocess have not been established yet. Here, we investigate the μ-dependent bioprocess performance of the model-acetogen *Clostridium autoethanogenum* in CO and syngas (CO + CO2+H2) grown chemostat cultures and assess systems-level metabolic responses using gas analysis, metabolomics, transcriptomics, and metabolic modelling. We were able to obtain steady-states up to μ ~2.8 day^{-1} (~0.12 h^{-1}) and show that faster growth supports both higher yields and productivities for

reduced by-products ethanol and 2,3-butanediol. Transcriptomics data revealed differential expression of 1,337 genes with increasing μ and suggest that *C. autoethanogenum* uses transcriptional regulation to a large extent for facilitating faster growth. Metabolic modelling showed significantly increased fluxes for faster growing cells that were, however, not accompanied by gene expression changes in key catabolic pathways for CO and H2 metabolism. Cells thus seem to maintain sufficient „baseline" gene expression to rapidly respond to CO and H2 availability without delays to kick-start metabolism. Our work advances understanding of transcriptional regulation in acetogens and shows that faster growth of the biocatalyst improves the gas fermentation bioprocess.

19. CHANGES IN HEMP GROWTH AND CONTENT OF CANNABINOIDS AFTER SEEDS TREATMENT WITH COLD PLASMA, VACUUM, AND ELECTROMAGNETIC FIELD

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One of the most important issues of sustainable agriculture is to increase crop productivity and yield without the use of pesticides. To achieve these goals, intensive research is currently being carried out on the use of cold plasma (CP) and electromagnetic field (EMF).

This study compared the response of two varieties of hemp (*Cannabis sativa*), 'Futura 75' and 'Santhica 27', to seed treatment with low-pressure condenser-type CP, dielectric barrier discharge CP (DBD), and EMF was compared. Vacuum treatment was used as an additional control for

low-pressure CP treatment. Changes in morphometry and cannabinoid content in leaves and inflorescences of plants have been studied.

The results showed that the effect of seed treatment with stressors on plant growth in the field depends on the variety of hemp. They confirmed previously obtained data [1] that CP suppresses, and EMF promotes the growth of 'Futura 75' variety. CP inhibited the growth of 'Santhica 27' plants, but DBD plasma significantly stimulated it. The positive effect of EMF was smaller compared to the effect on 'Futura 75' variety. Vacuum treatment of the seeds slightly improved the growth of both varieties and increased the cannabinoid content. Treatment protocols suitable for increasing CBD production in 'Futura 75' leaves have been identified: V3 and EMF2, in inflorescences – V2, V3 and DBD2; To increase CBD / CBG production in 'Santhica 27' leaves: V3, DBD1, DBD2, EMF2, in inflorescences – DBD1, DBD2, EMF3. There were no correlations between stress-induced changes in plant growth or changes in cannabinoid levels and changes in trichome density.

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20. THE EFFECT OF METFORMIN ON CULTURED MICROGLIA CELLS UNDER NORMOXIC AND MILD-HYPOXIC CONDITIONS

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Hypoxic brain injury may affect neural tissue via microglia activation, however, mechanisms and consequences of these processes during hypoxia are not fully elucidated yet. We aimed to investigate effects of anti-hyperglycemic agent – metformin on developing brain microglia cells under normoxic or mild- hypoxic conditions.

In this study primary rat microglial cultures (≥85 % microglia) at 7–11 DIV were treated with metformin (Met), cyclosporin (CsA) and rotenone (Ro). Cell cultures were incubated with or without pharmacological agents under normoxic and mild-hypoxic (93% N2, 5% CO2, 2% O2; 37°C) conditions for 24 h.

It was shown that mild hypoxia (2% oxygen) had no effect on microglial cell viability which remained above 90%. None of Met concentrations (0,1mM; 0,5mM and 3mM) had effect on viability and number of microglial cells. CsA under hypoxic conditions tended to decrease both – cell number and viability, while Ro has no effect on number and viability of cells. Mild-hypoxia increases glutamate in microglia culture media, and pre-treatment with Met but not Ro or CsA tend to reduce glutamate levels. We also found that none of the compounds effectively blocked mPTP opening in intact cells. Calcium dependent fluorescence measurements showed spontaneous calcium spikes; their generation was suppressed by CsA or trolox (0.1 mM), and enhanced by Ro, suggesting that Ca2+ spikes were mediated by mPTP opening. Hypoxia increased the frequency of Ca2+ spikes, while Met reduced the effect of hypoxia.

These results suggest that hypoxia facilitates opening of mPTP in monotypic cell cultures and may cause release of glutamate into culture medium which may be reduced by Met.

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21. THE DEVELOPMENT OF A SELENOPHENE- AND DEAZAPURINE- BASED PHOTOLUMINESCENT PROBE OF PROTEIN KINASES

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Protein kinases (PKs) are enzymes that catalyze the phosphorylation of proteins in organisms and thereby regulate many cellular processes. Their overexpression and dysregulation is associated with severe diseases, such as cancer and diabetes, therefore PKs have become one of the most studied targets in pharmaceutical research. Various inhibitors are being developed for the regulation and analysis of protein kinases.

Bisubstrate inhibitor design, wherein the inhibitor binds simultaneously to the ATP-binding site and peptide-binding area, provides higher affinity and selectivity compared to inhibitors that only bind to one of the aforementioned binding sites[1]. The bisubstrate PK inhibitors (ARCs) developed in the medicinal chemistry workgroup of the University of Tartu

are conjugates of an adenosine analogue and a peptide, connected by a linker. ARCs labelled with fluorescent dyes can be used as photoluminescent probes of PKs.

ARCs comprising a selenium heterocycle have exhibited intense long-lifetime photoluminescence when bound to PKs[2]. In the present work an ARC-type inhibitor ARC-1912 containing 7-deazapurine and selenophene moieties was developed. ARC-1912 binds strongly to the catalytic subunit of cAMP-dependent protein kinase (PKAc) with a K_D value in the low nanomolar range. The emission maximum of the phosphor-sensitized delayed fluorescence of ARC-1912-PKAc complex is red-shifted, compared to the emission spectra for previously reported selenium-containing ARCs. The probes such as ARC-1912 can be used for the development of assays for protein kinases that are based on the quantification of time-gated luminescence intensity (TGLI). These assays find applications for the biochemical characterization of novel protein kinase inhibitors as well as in diagnostic methods based on the detection of disease-related protein kinase biomarkers.

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22. TRANSLATIONAL FIDELITY IN THE ABSENCE OF Ψ32 AND Ψ38-40 IN THE ANTICODON STEM-LOOP OF TRNAS

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More than 150 different modifications have been described in RNA molecules and no class of RNAs contains more – both in number and diversity – chemical alterations than tRNAs. Different functionally important regions in tRNAs are clustered by pseudouridines (Ψ), however, its specific functions remain elusive. In our research group, two Ψ synthases, TruA and RluA, have been previously shown to affect mutation frequency in exponentially growing cells [1]. TruA and RluA isomerize uridine to

pseudouridine in the tRNA anticodon stem-loop (ASL), in positions 38-40 (TruA) and 32 (RluA). Modifications in the ASL have been previously shown to affect the fidelity of translation [2]. Therefore, it is possible that the change in mutation frequency seen in the absence of these Ψ synthases is a result of decreased accuracy of translation.

Although mistranslation has been investigated previously, to our knowledge, there have been no studies published comparing mistranslation in different species. By using a dual-luciferase reporter assay we measured the translational fidelity in both wild-type and TruA- and RluA-deficient strains of different bacterial species. Our results point towards great diversity in terms of translational fidelity not only between different species of bacteria but also depending on the genetic context. The absence of TruA affects translational fidelity in some of the investigated genetic contexts, however the effect varied between different species, while the lack of RluA had no effect. Whether this points towards another mechanism linking Ψ-synthases to mutations or just shows that the link is not due to changes in global translational fidelity, but rather specific and discrete events, needs further research.

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23. ISCHEMIA/REPERFUSION AND CAFFEIC ACID PHENETHYL ESTER EFFECT ON KIDNEY MITOCHONDRIA *IN VIVO*

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Kidney ischemia/reperfusion (I/R) injury is a complex process closely associated with mitochondrial damage and free radical generation [1]. Nowadays antioxidants have become the focus of interest to treat various pathologies, including ischemia/reperfusion injury. Caffeic acid phenethyl ester (CAPE) is a polyphenolic antioxidant, an active component of bee glue [2]. The aim of this study was evaluate whether CAPE can protect rat kidney mitochondria from *in vivo* ischemia/reperfusion-induced injury. Rats were injected into the tail vein with CAPE (22 mg/kg of rat body). Warm kidney ischemia in rats was induced by the clips around artery for 20, 30, 40 and 60 min. Reperfusion was performed for 30 min. Mitochondria from rat's kidneys were isolated by the method of differential centrifugation and used for further investigations. Ischemia/reperfusion alone caused significant alternations in mitochondrial respiration, decreased Complex I and succinate dehydrogenase activities and induced necrotic cell death. CAPE protected the glutamate/malate oxidation and Complex I activity and diminishes ischemia/reperfusion-induced LDH release protecting from necrotic cell death. Our results showed, that CAPE has no protective effects on succinate oxidation or Complex II+III activity, but partially protects succinate dehydrogenase from ischemia/reperfusion-induced damage. In summary, CAPE protects the kidney mitochondria from ischemia/reperfusion, and shows potential as a promising mitochondria-targeted antioxidant for the development of pharmaceutical preparations against oxidative stress-related diseases.

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24. (SWEET)MULTIBAC(MAM) LIBRARY FOR FRIZZLED RECEPTORS STUDIES WITH FLUORESCENCE SPECTROSCOPY AND MICROSCOPY

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The family of Frizzled receptors (FzdRs) provides a broad and practically untapped source of potential targets for therapeutic interventions. It consists of ten FzdR subtypes and upon activation by endogenous WNT ligands they trigger multiple intracellular signal transducing proteins, such as Dishevelled scaffold protein or heterotrimeric G proteins. Surprisingly little is known about the selectivity, affinity and the efficacy of pharmacological compounds directly targeting FzdRs, and this originates from the long-standing difficulties to obtain recombinant, pure and biologically active WNTs. In addition, the study of different G-protein activation pathways triggered by FzdR has been very limited due to the lack of adequate experimental tools.

We have found that some of the above-mentioned obstacles could be overcome by using a Baculovirus–mediated multigene DNA cargo delivery system – (Sweet)MultiBac(Mam)[1]. This system is very useful for the efficient expression of multiprotein complexes in both insect and mammalian cells. It allows the generation from insect cells Budded Baculovirus particles that display receptors on their surface as well the production of virus-like particle from mammalian cells that also contain proteins of interest. Those biological nanoparticles are an excellent source of target proteins for ligand-receptor binding studies with assays such as our previously published fluorescent anisotropy (FA)[2] or total internal reflectance fluorescence microscopy (TIRFM)[3].

We also provide examples where, with baculovirus it is possible to express in the same mammalian cells some FzdRs, heterotrimeric G-proteins and biosensors that measure certain activation signals (in particular cAMP level). To cover the whole range of G protein activation pathways, we use the expression of diferent chimeric Gs subunits (GsX)[4],

which allow rewiring various activation signals into an increase of the intracellular cAMP concentration.

We believe that presented library would be very useful for future studies on FzdR.

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25. QUANTIFICATION OF ALPHAVIRAL VECTORS USING DROPLET DIGITAL PCR

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Alphaviruses are enveloped (+) ssRNA viruses that are thought to be promising vectors for gene therapy. Quantification of the viral titer is essential for gene therapy studies. Quantitative real-time PCR (qPCR) is widely used to determine the genome titre. The approach, however, requires the use of a standard curve and is sensitive to PCR inhibitors. Furthermore, genome titres cannot predict the infectivity of the virus and, therefore, cannot be used to determine the multiplicity of infection (MOI) during the experiments. Quantification of the infectious titer can be achieved using immunostaining of infected cells. However, the specific antibodies for modified viral vectors are not always commercially available.

To address these limitations, we have developed the method for the quantification of alphaviral vectors using droplet digital PCR (ddPCR). Recently ddPCR was shown to successfully quantify adeno-associated viral vectors [1]. The method does not require a standard validation curve and is less sensitive to PCR inhibitors. As a model virus replication-deficient recombinant Semliki Forest virus (SFV) encoding fluorescent protein genes (EGFP or DS-Red) was used as its concentration can be easily calculated using fluorescent microscopy of infected cells. Quantification of the genome titer was optimised using a virus concentration gradient. The method demonstrated stability and reproducibility using different sets of primers

targeting several parts of the SFV genome. Moreover, the genome concentration in the combination of two viruses was successfully determined by adjusting annealing/extension temperature and virus:virus ratio. Finally, to quantify the infectious titer, we performed single cell ddPCR previously described to analyse the ratio of genetically modified cells after stem cell gene therapy [2]. The data obtained in this study indicated that ddPCR can be used to quantify both the genome and the infectious titers of the Semliki Forest virus.

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26. EXPRESSION OF "THE BLUE GENE", FLAVONOID 3', 5'-HYDROXYLASE FROM *VACCINIUM* SPECIES IN THE YEAST *PICHIA PASTORIS*

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Flavonoids are polyphenolic compounds found in plants. They are precursors for anthocyanins – pigments that give the flowers and fruits red, purple and blue colouration. Flavonoid 3', 5'-hydroxylases (F3'5'H, EC 1.14.14.81) belong to the cytochrome P450 superfamily and they introduce hydroxyl groups at the 3' and 5' positions of the B-ring of flavonoids, thereby directing the anthocyanin synthesis pathway from the orange and reddish-purple pelargonidin and cyanidin type anthocyanins to purple-blue delphinidins. Berries of *Vaccinium* species plants contain high levels of anthocyanins and therefore, bilberry (*Vaccinium myrtillus*) and bog bilberry (*Vaccinium uliginosum*) were chosen as potential sources of F3'5'H genes. Obtaining the sequences encoding bilberry F3'5'H (VmF3'5'H) and bog bilberry F3'5'H (VuF3'5'H) involved RNA extraction from ripe berries picked from Western Estonia, design of degenerate primers, complementary DNA (cDNA) synthesis and rapid amplification of cDNA ends. The open reading frames of both F3'5'H sequences consisted of 1539 base pairs encoding 512 amino acid residues that were 95%

identical (GenBank accession numbers OK533468 and OK533469). Partial sequences of other isoforms were also detected. The yeast *Pichia pastoris* (*Komagataella phaffii*) was transformed with the VmF3'5'H and VuF3'5'H sequences encoding a hexahistidine tag at their N- or C-terminus. After 48 hours of methanol induction the cell lysates were subjected to western blotting. The proteins fused with an N-terminal affinity tag were hardly detectable. Modest amounts of F3'5'H fused with a C-terminal hexahistidine tag were detected whereas the amount of VuF3'5'H was slightly higher. As expected, the proteins were in the membrane pellet fraction, indicating that *Vaccinium* F3'5'Hs are membrane proteins. The study expands the pool of F3'5'H proteins with potential future application in biotechnological anthocyanin synthesis.

27. QUANTIFICATION OF BETA-LACTAM ANTIBIOTICS IN HUMAN PLASMA BY HPLC-MS/MS METHOD FOR THERAPEUTIC DRUG MONITORING

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β-lactam antibiotics are the cornerstone of antibacterial treatment and frequently prescribed drugs, especially in the intensive care units (ICU) of hospitals. Contemporary β-lactam antibiotic dosing is debatable in severely ill patients, since the occurrence of pathophysiological changes in critical illness can result in great inter-individual variability. Therapeutic drug monitoring (TDM) is a commonly used dosing strategy to optimize exposure and thereby minimize toxicity and maximize the efficacy. Currently, TDM of β-lactam antibiotics is rarely performed, due to poor availability in clinical practice. We describe here simple and rapid HPLC–MS method for the determination of ampicillin, amoxicillin, cefepime, ceftazidime, imipenem, meropenem, cilastatin and piperacillin in human plasma. This method involves simple sample preparation steps and was comprehensively validated according to EMA guidelines [1]. For all analytes, mean accuracy and precision values were within the acceptance value. The lower and upper limits of quantification were found to be

sufficient to cover the therapeutic range for all antibiotics. Developed simple, sensitive and rapid assay was implemented in clinical laboratory of the North Estonia Medical Centre to promote the TDM of β-lactam antibiotics. The feasibility of the analytical procedure was demonstrated by therapeutic monitoring of meropenem in six patients with and without acute renal failure.

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28. ANTIOXIDATIVE AND ANTI-*BORRELIA* ACTIVITY OF PHYTOCHEMICALS IN *RUBIACEAE* SPECIES

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Lyme disease, caused by the spirochaete *Borrelia burgdorferi*, is the most common vector-borne disease in Europe[1]. The chronic illness can affect joints and muscles, nervous and cardiovascular systems, and withstand several rounds of antibiotic treatment[2]. The recalcitrance of the infection is tied to the latent bacterial forms[3]. Therefore, novel therapeutic approaches are required to combat the *B. burgdorferi* persisters. With the aim of discovering new lead compounds with distinct anti-*Borrelia* activity, the antioxidative and antibacterial properties of several plants found in Estonia have been demonstrated by our group. The presented research focuses on plants from the *Rubiaceae* family, *Galium verum*, *Galium aparine* and *Galium mollugo*. The main groups of bioactive compounds in the plants were quantified by colorimetric tests: total flavonoids by the $AlCl_3$, total iridoids by the Trim-Hill, and total polyphenols by the Folin-Ciocalteau method. The antioxidative activity of all extracts was evaluated using the $ORAC_{FL}$ method. To identify and quantify the main phytochemicals in the extracts, the comparative fingerprinting analysis of all species was performed using both CE-DAD and HPLC-DAD. The anti-*Borrelia* activity of the plant extracts was tested on the latent bacterial forms using the SYBR

Green I/PI assay. To identify the antibacterial phytochemicals, the active extracts were fractionated and tested on *B. burgdorferi*, and the active extracts and fractions were chemically characterized by HPLC-DAD-MS/MS. The cytotoxicity of active extracts and fractions was evaluated on mammalian cells by both 48h end-point measurements using WST1 assay, and real-time measurements during 48h using RealTime-Glo™ MT Cell Viability Assay.

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29. THE SECOND LIFE OF MANDARINE POMACE: A VALUABLE SOURCE OF BIOACTIVE COMPOUNDS

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Citrus fruits (including mandarins) are one of the most extensively cultivated crops. Citrus contains a range of highly beneficial bioactive compounds, such as essential oils, polyphenols, carotenoids, and vitamins. On consumption or processing, approximately 60% of the fruit remains as inedible waste, which includes peels, seeds, pulp, and segment residues. These co-products, without proper treatment and disposal, might cause negative environmental impacts. At the same time, this waste still consists of substantial quantities of bioactive compounds, which makes it ideal sustainable and renewable resource for obtaining high added-value products for food and pharmaceutical industries.

The aim of the current research was to investigate the parameters and effects of the implementation of sequential processes on the selective recovery of limonene, hesperidin and other high-value molecules. The sustainable and effective extraction techniques, such as steam distillation

for limonene extraction and ultrasound-assisted extraction (UAE) for hesperidin isolation, were extensively evaluated, separately and sequentially, to find out the optimal hyphenation conditions. At current stage of the research main emphasis was focused on selective extraction of hesperidin by addition of sodium carbonate to primary biomass prior to distillation process. The influence of salt concentration on limonene as well as hesperidin yield was investigated. Additionally, for UAE optimization, three process independent variables – temperature, time and power of UAE – were evaluated. The Response Surface Methodology was utilized for determination of the optimal extraction conditions that provided the maximum hesperidin content.

30. CREATION OF VIRUS-LIKE PARTICLE-BASED THERAPEUTIC VACCINE CANDIDATE AGAINST ALZHEIMER'S DISEASE

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Alzheimer's disease is the prevailing cause of dementia, affecting about 30 million people worldwide. The main causes of Alzheimer's disease are believed to be extracellular accumulation of Aβ peptide in the form of amyloid plaques and intracellular accumulation of tau protein as neurofibrillary tangles in the brain. Most of the plaque forming Aβ peptides end after residue 42 and are formed by cleavage of APP with β and γ secretases. Currently there is no available medication to prevent, reverse or stop the progression of disease and treatment is only symptomatic. Various monoclonal antibodies targeting Aβ peptides have been proposed for treatment of Alzheimer's disease. Now the Eli Lilly company is developing *Donanemab*, which recently displayed promising results in Phase II studies. *Donanemab* targets pyroglutamate form of Aβ(p3-42) peptide, which is aggregated in amyloid plaques, but absent in physiological fluids. However, it is possible to force the organism itself to produce auto-antibodies against Aβ(p3-42) peptide plaques, which in principle should work in a similar way to *Donanemab* at a fraction of cost. An efficient way to induce auto-antibodies is to display the respective antigens on a surface of suitable carriers, such as virus-like particles (VLPs).

We coupled a 6 amino acids long peptide, corresponding to residues 3-8 of APP protein, to VLPs by genetic fusion. Himeric particles were co-produced with human glutaminyl cyclase thereby in the purification process, the first amino acid of displayed epitope was pyroglutaminilated. The obtained VLPs were tested for their immunogenicity in mice models. In future studies we will test the effectiveness of the vaccine in transgenic mouse models.

31. MARAFIVIRUS COAT PROTEIN VARIANTS' EXPRESSION IN BACTERIAL SYSTEM FOR VIRUS-LIKE PARTICLE SELF-ASSEMBLY

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Plant viruses included in the genus Marafivirus are small, isometric plant viruses with a monopartite ssRNA(+) genome varying from 6.3 kb to 7.1 kb in length (excluding polyA tail)[1]. Coat protein (CP) is coded as a single domain but expressed in two forms – minor and major. The ratio between both forms in a native virus is 1:3, minor vs major[2]. We developed expression constructs for both forms of a newly discovered marafivirus - sea buckthorn marafivirus (SBuMV) to test both CPs for virus-like particle (VLP) self-assembly after expression in the bacterial expression system and also to test in co-expression of both forms to evaluate the Minor (p31) and major (p21.2) CPs impact upon the formation of VLPs.

p31 and p21.2 CPs were expressed in the *Escherichia coli* expression system and were soluble. p31 was expressed in smaller amounts if compared with p21.2. CPs were purified by sucrose gradient and sucrose cushion. TEM analysis of purified CPs revealed that p31 formed protein aggregates, but p21.2 was self-assembled in VLPs of around 30 nm in diameter.

To find out if p31 has any influence over the formation of VLPs, we performed two types of co-expression experiments. In one case p21.2 and p31 were expressed from the same plasmid, in the other – from two different plasmids, where p21.2 was expressed from a medium copy number plasmid and p31 – from a low copy number plasmid. Depending on the ratio of p31, VLP self-assembly was disturbed.

Our experiments revealed that for VLPs self-assembly p31 isn't required. Co-expression experiments of p31 and p21.2 revealed that VLPs self-assembly is influenced in ratio independent manner.

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32. IMPACT OF *TOTIVIRIDAE* L-A DSRNA VIRUS ON *SACCHAROMYCES CEREVISIAE* HOST: TRANSCRIPTOMIC AND PROTEOMIC APPROACH

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Totiviridae L-A virus is a persistent *Saccharomyces cerevisiae* dsRNA virus. It encodes the major structural capsid protein Gag and Gag-Pol fusion protein, responsible for virus replication and encapsulation. These features also enable the copying of satellite dsRNAs (called M dsRNAs) encoding a secreted toxin and immunity to it (known as killer toxin). Viral capsid pore presumably functions in nucleotide uptake and viral mRNA release. During cell division, sporogenesis, and cell fusion, the virions remain intracellular and are transferred to daughter cells.

By employing high throughput RNA sequencing data analysis, we describe the influence of solely L-A virus on expression of genes in three different *S. cerevisiae* hosts. We provide a new perception into *Totiviridae* L-A virus-related transcriptional regulation, encompassing multiple bioinformatics analyses. Transcriptional responses to L-A infection were

similar to those induced upon stress or availability of nutrients. It also delves into the connection between the cell metabolism and L-A virus-conferred demands to the host transcriptome by uncovering host proteins that may be associated with intact virions. To better understand the virus-host interaction, we applied differential proteomic analysis of virus particle-enriched fractions of yeast strains that harbor either complete killer system (L-A-lus and M-2 virus), M-2 depleted or virus-free. Our analysis resulted in the identification of host proteins, associated with structural proteins of the virus (Gag and Gag-Pol).

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33. MODULATING MICROGLIA PHAGOCYTOSIS

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Ischemic stroke is one of the leading causes of death and disability in adults. Currently, there are no effective drugs to promote the functional recovery from stroke. Adequate healing of the damaged brain area depends on clearance of cell and myelin debris, but this process is slow and perturbs with neuronal regeneration. Thus, enhancing phagocytosis could improve removal of cell debris. We have created a small library of lentiviral vectors (LVs) encoding different genes related to phagocytosis or recruitment of microglia/macrophages, namely Monocyte chemoattractant protein 1 (MCP1), three isoforms of Macrophage colony-stimulating factor (M-CSF), Complement Component 3 (C3), Complement Component 3a (C3a), Adhesion G protein-coupled receptor E1 (Emr1/ADGRE1/F4/80), MER receptor tyrosine kinase (MerTK) and Mesencephalic astrocyte-derived neurotrophic factor (MANF). Their effect on phagocytosis and induction of inflammation were tested in microglia (BV2) after transient transfection in vitro by phagocytosis assay and cytokine (TNFa, IL-6 and IL10) ELISAs. The highest effect on phagocytosis was detected with LV-MerTK, LV-MCSF32-E and LV-MCSF1-E while LV-C3a and LV-Emr1 transfections enhanced phagocytosis over 80% of the induction of the positive control. In parallel, LV-MANF and LV-MerTK were equally potent in enhancing TNFa and IL-6.

In summary, this is the first study to compare the effect of different chemotactic and phagocytosis related proteins enhancing phagocytosis and changing the inflammatory profile of microglia.

34. OXYFUNCTIONALIZATION STUDY OF AROMATIC COMPOUNDS BY BIOCATALYTIC AND CHEMICAL METHODS

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Oxygenation reactions encompass numerous synthesis applications, though the most commonly used chemical methods require strong oxidants and are poorly selective. Previously, it was shown that soluble di-iron monooxygenase PmlABCDEF (PML) is an attractive biocatalytic option to access oxyfunctionalized compounds[1]. This work aims to investigate the Pml-catalyzed bioconversions of the allyl-substituted aromatic substrates into highly-regarded epoxide derivatives. The various allyl-substituted aromatics were oxidized both chemically using *meta*-chloroperoxybenzoic acid and employing PML monooxygenase to test whether the enzyme is inherently stereoselective. All biocatalytic reactions were performed using *Pseudomonas putida* KT2440 bacterial strain harboring a recombinant plasmid containing the *pmlABCDEF* gene. Also, combining chemical and biocatalytic methods could be an attractive new platform to synthesize compounds that would be difficult to obtain by chemical synthesis alone.

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35. *GEOBACILLUS* SP. 95 ESTERASE: ANALYSIS OF IMPORTANT AMINO ACIDS FOR SUBSTRATE BINDING AND ACTIVITY

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Both the industry, and the natural environment are complex systems, which, if not maintained, could have enormous repercussions on the global scale. Improvements can be made from the smallest parts of these systems, and even small changes, when accumulated, could make an impact by both improving industrial processes, reducing their costs and most importantly their environmental effect. Enzymes are one such improvable part. Knowledge of the fundamental functions of enzymes can help expand their industrial applications.

Based on earlier analysis[1] we performed mutagenesis of *Geobacillus* sp. 95 esterase (GDEst-95) to hopefully improve its catalytic properties, as well as gain important insights into the structure-function relationship of lipolytic enzymes as a whole.

To analyse the importance of candidate amino acids site-directed mutagenesis of GDEst-95 was performed using overlap extension method. The resulting mutant genes were transformed into recombinant cloning (pJET1.2blunt + *E. coli* DH5α) and expression (pET21c (+) + *E. coli* BL21 (DE3)) systems. Protein synthesis was induced using IPTG, and purification was performed using affinity chromatography. Activity of mutated GDEst-95 esterase variants was measured using p-NP esters as substrates.

Our analysis of candidate amino acids (Thr317, Leu226, Leu411) shows that these amino acids are important for the activity of GDEst95. Based on earlier analysis these amino acids are localized close to the substrate binding site, and their mutations impact both the activity and substrate specificity of GDEst95.

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36. ENGINEERED DNA METHYLTRANSFERASE DNMT1 FOR SELECTIVE COVALENT TAGGING OF METHYLATION SITES IN LIVE CELLS

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DNA methylation of cytosine in CpG dinucleotides to 5-methylcytosine is a fundamental epigenetic mechanism involved in mammalian development and disease. For a deeper understanding of these processes, we engineered AdoMet-dependent mouse DNA methyltransferase Dnmt1 to enable catalytic transfer of extended moieties onto DNA from a synthetic cofactor analog, Ado-6-azide, *in vitro*. Then, we edited the *Dnmt1* locus in mouse embryonic stem cells to install the engineered codon using CRISPR-Cas9 genome editing system. Engineered Dnmt1 and pulse internalization of the Ado-6-azide cofactor by electroporation permitted us to enable selective covalent tagging and precise mapping of catalytic Dnmt1 targets in mammalian cells.

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37. INVESTIGATION OF GENES AND ENZYMES INVOLVED IN THE METABOLISM OF PYRAZINE DERIVATIVES

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Pyrazines are volatile compounds with a six-membered aromatic ring containing two nitrogen atoms. Pyrazines have a wide range of applications in the food industry and are also used as pharmaceuticals, insecticides, and pesticides[1]. Pyrazine-*N*-oxides are no exception. They are active biological compounds with applications in the aforementioned fields[2].

The aim of this work is to develop an environmentally friendly platform for the synthesis of pyrazine *N*-oxides. We have shown that using whole cells of *Pseudomonas putida* KT2440 producing recombinant L-threonine-3-dehydrogenase and PmlABCDEF monooxygenase, it is possible to produce 2,5-dimethylpyrazine-*N*-oxides (2,5-DMP-*N*-oxides) from L-threonine. Attempts are being made to develop a more efficient platform for this synthesis. The first goal is to construct a genetically modified *Pseudomonas putida* KT2440 strain that would be capable of realizing elevated levels of L-threonine in the cells. Thus, based on the metabolic pathways of the organism, certain reactions have been identified for which the responsible genes should be removed (markerless deletions) in order to reduce the number of possible side reactions in L-threonine biosynthetic pathway. To enhance the expression of certain genes involved in the biosynthetic pathway, responsible genes are amplified from the genome of the *Pseudomonas putida* KT2440 itself by PCR, integrated into plasmids, and introduced to the cells. Such a genetically modified *P. putida* KT2440 strain should ensure the successful synthesis of 2,5-DMP-N-oxides from renewable carbon sources (glucose, glycerol, etc.).

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38. OPTIMIZATION AND APPLICATION OF STABLE ISOTOPE MASS SPECTROMETRY METHOD TO STUDY BREAST CANCER ENERGY METABOLISM

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The aim of given research was to investigate breast cancer metabolism by optimizing and utilizing ^{18}O stable isotope-based Gas Chromatograph-Mass Spectrometry (GC-MS) method. A liquid chromatography method was used to separate and quantify metabolites from cell lines of control (MCF10A) and breast cancer subtypes Luminal A (MCF-7) and triple negative (MDA-MB-231). The ^{18}O-labelled metabolites were analyzed in GC-MS for changes in energy metabolism of cancer cells compared to control.

The study indicates that the GC-MS method is suitable for breast cancer energy metabolism research. The intracellular ATP reserves were substantially larger in Luminal A and triple negative cell lines than that of control. In contrast the phosphocreatine (PCr) levels, mitochondrial ATP synthesis rates (γ-ATP[^{18}O]), Adenylate Kinase (AK) phosphotransfer rates (β-ATP[^{18}O]) and Creatine Kinase(CK) fluxes (PCr[^{18}O]) were notably lower in cancer cells compared to control. The results gave reasonable basis to conclude that contributions in intracellular energy production by mitochondrial ATP synthesis, CK and AK metabolic pathways have been altered in cancer cells. The high ATP levels in cancer cells may indicate upregulation of glycolytic pathway[1].

In conclusion, the study suggests that evaluation of intracellular ATP levels may be a good diagnostic marker for breast cancer. More complex analyses should be considered in the future, involving the measurements of phosphotransfer enzymatic fluxes among determining the expression level of their isoforms and measuring glycolytic pathways. Such comprehensive studies could offer more deep understanding about breast cancer energy metabolism at different stages and in distinct breast cancer subtype by finding the link between phosphotransfer enzyme expression patterns and their enzymatic fluxes.

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39. THE BIOCHEMICAL CHARACTERIZATION OF *THERMUS THERMOPHILUS* LARGE LACCASE IN RELATION TO THE VALORIZATION OF LIGNIN

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Lignin is the second-most abundant component of plant material. It is a complex organic polymer that plays a key part in providing the structural support of most plants.[1] The foremost commercial production of lignin comes from paper making. The precursor of paper is lignocellulose, roughly ⅓ of which is lignin. Right now, most of it is burned as fuel, while only a small fraction is valorized.

Our main goal is to find alternative solutions for lignin valorization. Utilization of lignin degrading enzymes is one of the options for lignocellulosic biomass conversion and lignin valorization.

Thermus thermophilus is a Gram-negative bacterium that is an extreme thermophile and has been found to be important in the degradation of organic materials in the thermogenic phase of composting.[2] Laccases (LLAC) are multicopper oxidases that oxidize a variety of phenolic substrates. They are classified as lignin-modifying enzymes since they take part in both formation and degradation of lignin.

In this study we focus on the large laccase from the bacteria *T. thermophilus* (*Tt*LLAC). We have successfully expressed and purified recombinant *Tt*LLAC protein. Kinetic parameters have been determined for two classical laccase substrates. The enzyme retained the thermophilic properties of the bacterium and stayed fully active for at least 48 h at 70 °C. High quality crystals were also obtained and analyzed using X-ray crystallography. *Tt*LLAC mediated lignin degradation is demonstrated for pine, aspen and barley straw organosolv lignins.

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40. MICROGLIA IMMUNOMETABOLISM CHANGES AFTER VIRUS MIMETIC POLY I:C-PRIMED HUMAN AIRWAY EXOSOME-LIKE VESICLES UPTAKE

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Introduction: Viral infections of upper respiratory tract can induce airway epithelial exosome-like vesicles (ELVs) with inflammatory cargo. ELVs can easily cross blood brain barrier; thus, they might transmit inflammatory signal from the periphery to the brain. Our previous *in vivo* studies with rodents revealed that virus mimetic-primed airway ELVs are internalised by microglia, resulting in induced inflammatory changes. The aim of this study – to investigate the effect of virus mimetic poly I:C-primed human bronchial epithelial cell ELVs on human microglia cell (HMC).

Methods: Poly I:C primed- and not-primed airway ELVs were isolated from culture medium by polymer precipitation and were characterized by particle size (Zetasizer) and specific markers (ELISA). Poli I:C encapsulation in ELVs was determined by dihydrorhodamine-conjugated poly I:C. The uptake of ELVs by HMC was observed with fluorescent microscopy. Mitochondrial function of HMC was determined with *Seahorse XFp* analyzer. Intracellular ROS and mitochondrial superoxide were determined with 2',7'-dichlorofluorescein diacetate and MitoSOX™ Red, respectively. The caspase-1 activity was assessed by luminescent assay. RT-PCR was used to determined inflammatory gene expression. Statistical data analysis was performed by *IBM SPSS Statistics 20* software.

Results: Mostly, EVs were 37-58 nm and CD9, CD63, CD81 markers were determined. ELVs were internalized by HMC after 2 hours treatment. Mitochondrial function of HMC was significantly inhibited and glycolysis activated after 24-hour treatment with primed- ELVs. This was accompanied by increase in the production of intracellular and mitochondrial ROS, also, by inflammasome activation and increase of *IL-6*, *TNF-α* and *PTGS2* expression. Such effects were not observed in HMC cultures treated with not-primed ELVs.

Summary: Microglial cells after poly I:C-primed ELVs uptake, undergo immune-metabolic transition characterised by shift from mitochondrial to glycolytic ATP production. Also, increase in ROS and pro-inflammatory cytokines production.

41. PRE-SOWING TREATMENT OF COMMON BUCKWHEAT SEEDS WITH COLD PLASMA AND ELECTROMAGNETIC FIELD RESULTS IN STIMULATED SEEDLING GROWTH AND INCREASED GRAIN HARVEST

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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 maximal seed germination and germination rate *in vitro* and emergence in the substrate (in cassettes). After 5 weeks of cultivation in the greenhouse, the seedlings grown in the cassettes were harvested, their morphometric analysis was performed and organic acids in root exudates were detected. 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%. Only succinic

acid was found in the root exudates. All treatments except the V5 group reduced the amount of succinic acid per 1 g of root tissue in the root exudates compared to the control. 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 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. 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.

42. GENOME WIDE ANALYSIS OF CIRCULATING MIRNAS IN GROWTH HORMONE SECRETING PITUITARY NEUROENDOCRINE TUMOR PATIENTS' PLASMA

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Objective: Circulating plasma miRNAs have been widely studied in the field of cancer research however they remain understudied in field of Pituitary neuroendocrine tumor (PitNET) research. The aim of this study was to discover circulating plasma miRNAs in plasma of PitNET patients with a focus on GH secreting PitNETs using NGS approach and evaluate

how the expression levels of selected miRNA candidates are impacted by somatostatin analogue (SSA) treatment and by different PitNET subtypes.

Methods: Whole miRNA sequencing of pre- and postoperative plasma samples from GH and NF PitNET patients was done on Illumina MiSeq and NextSeq 500 platforms. Reads were mapped to miRBase V22 using CLC Genomics Workbench and differential expression analysis was done on DESeq2. Using RT-qPCR miRNA candidates were then tested in longitudinal manner in GH PitNET patients receiving SSA treatment and in group consisting of GH vs. NF PitNET patients.

Results: A total of 16 differentially expressed miRNAs (DEMs) were identified in plasma of GH secreting PitNEt patients 24 hours after surgery and by comparing GH secreting PitNET plasma against NF PitNET plasma we identified 19 DEMs. From these results seven miRNAs were selected for further testing by RT-qPCR of which miR-625-5p, miR-503-5p miR-181a-2-3p and miR-130b-3p were significantly downregulated in GH secreting PitNET patients receiving SSA treatment for one month. miR-625-5p also showed a significant decrease in plasma of GH secreting PitNETs compared to NF PitNETs.

Conclusions: Plasma levels of miR-625-5p, miR-503-5p, miR-181a-2-3p and miR-130b-3p are impacted by SSA treatment and miR-625-5p can potentially distinguish GH PitNETs from NF PitNETs. Therefore, further research of these markers in context of GH secreting PAs in warranted.

43. BACTERIAL EXPRESSION SYSTEMS BASED ON POTATO VIRUS Y-LIKE PARTICLES FOR VACCINE GENERATION

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Plant virus-like particle (VLP)-based vaccines have been studied for years, demonstrating their potential as antigen-presenting platforms. We describe the development and comparison of simple Escherichia coli-based antigen display platforms for the generation of potato virus Y (PVY) VLP-derived

vaccines, allowing the production of vaccines from single bacterial cell culture. We constructed four systems with the major cat allergen Fel d 1, namely, direct fusion with plant virus PVY coat protein (CP), mosaic PVY VLPs, and two coexpression variants of conjugates (SpyTag/SpyCatcher) allowing coexpression and conjugation directly in E. coli cells. For control experiments, we included PVY VLPs chemically coupled with Feld1. All constructed PVY–Feld1 variants were well expressed and soluble, formed PVY-like filamentous particles and were recognized by monoclonal Feld1 antibodies. Our results indicate that all vaccine variants induced high titers of anti-Feld1 antibodies in murine models. Mice that were immunized with chemically coupled Feld1 antigen exhibited the highest antibody titers and antibody–antigen interaction specificity as detected by binding avidity and recognition of native Fel d 1. IgG1 subclass antibodies were found to be the dominant IgG class against PVY-Feld1. PVY CP-derived VLPs represent an efficient platform for the comparison of various antigen presentation systems to help evaluate different vaccine designs.

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44. DETECTION OF SARS-COV-2 IN CLINICAL SAMPLES IN <10 MIN USING SUPERSCRIPT IV RT-LAMP MASTER MIX

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The global crisis caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) demonstrated a massive demand for fast pathogen detection and surveillance methods. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a technology that allows fast and specific viral RNA amplification in 5–30 minutes under isothermal conditions. Typical

RT-LAMP reactions include 4–6 target-specific primers, which facilitate formation of loop structures and promote faster amplification, a reverse transcriptase for reverse transcription (RT) of RNA to cDNA, and a DNA polymerase with strong strand-displacing activity. Target amplification is performed at a constant temperature (e.g., 65°C), hence simple equipment such as a heat block can be used for incubation of reactions.

Here we demonstrate the use of Invitrogen™ SuperScript™ IV RT-LAMP Master Mix and a custom-designed RT-LAMP primer set for fast detection of SARS-CoV-2 RNA following RNA purification from clinical samples. Average time-to signal was less than 10 minutes regardless of RNA input amount. The current protocol demonstrates compatibility of SuperScript IV RT-LAMP Master Mix with real-time fluorescence detection using Invitrogen™ SYTO™ 9 stain. Also demonstrated is endpoint detection, which includes visual evaluation of a color change in the reaction mix using

Invitrogen™ SYBR™ Green I stain or visual evaluation of RT-LAMP products using Invitrogen™ E-Gel™ precast gels.

45. METABOLIC PLASTICITY SUPPORTS COLORECTAL CANCER PROGRESSION

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Colorectal cancer (CRC) ranks as the 2nd highest cause of cancer death globally. It mostly develops from polyps which are growths on the inner lining of the colon or rectum. Cancer cells reprogram their energy metabolism to promote unregulated proliferation and growth. Recently, a metabolic combination of oxidative phosphorylation and glycolysis has been shown in several cancer types. Our results demonstrate that while CRC cells increase the activity of glycolysis, most of the ATP is still derived from OXPHOS[1].

While most of the cancer research focuses on cancer alone, we are also studying the energy metabolism of polyps. Combining the information we know about healthy tissue, polyps and tumors will help us to figure out the

mechanisms behind the reprogramming of energy metabolism during tumorigenesis. To understand the complex structure of cancer cells, we characterize ATP-synthesis by OXPHOS using high-resolution respirometry and detect the expression levels of several genes that are important in metabolic pathways.

Our most recent results measured by the high-resolution respirometry indicate that the activity of the glycolytic pathway increases in colon polyps. This phenomenon is also supported by the high expression of HKs, LDHA, GLUTs, and MCTs which all support aerobic glycolysis. Polyps also display increased expression levels of genes that are part of energy transfer pathways – AKs and CKs. Furthermore, while functional studies show that in tumors most of the energy is provided by OXPHOS, gene expression levels of the subunits of mitochondrial respiration chain complexes are considerably higher in polyps. These results suggest that in a premalignant polyp state, cells may increase several different pathways to adapt to the new environment and provide enough energy for rapid proliferation whereas in CRC cells energy production is rather well regulated.

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46. THE EFFECTS OF COMPLEX I INHIBITORS ON MITOCHONDRIAL PERMEABILITY TRANSITION AND ISCHEMIC BRAIN INJURY

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Ischemic brain injury is among leading causes of human deaths and disabilities. Damage to cerebral mitochondria, particularly opening of mitochondrial permeability transition pore (mPTP), is attributed as a key mechanism of ischemia-induced brain injury and cell death. Therefore,

modulation of mPTP may be a potential target for a disease-modifying treatment strategy in ischemic brain pathologies. Several studies have shown that mPTP may be modulated by mitochondrial complex I activity. The aim of this study was to investigate whether biguanides – metformin and phenformin as well as other inhibitors of Complex I of the mitochondrial electron transfer system may protect against ischemia-induced cell death in brain slice cultures by suppressing mPTP, and whether the effects of these inhibitors depend on the age of animals. The research was performed on brain slice cultures and isolated mitochondria from 5-7 days (premature) and 2-3 months old (adult) rat brains. In premature brain slice cultures, simulated ischemia (hypoxia plus deoxyglucose) induced necrosis whereas in adult rat brain slice cultures necrosis was induced by hypoxia alone and was suppressed by deoxyglucose. Phenformin prevented necrosis induced by simulated ischemia in premature and hypoxia-induced – in adult brain slices, whereas metformin was protective in adult brain slices cultures. In premature brain slices, necrosis was also prevented by Complex I inhibitors rotenone and amobarbital and by mPTP inhibitor cyclosporine A. The latter two inhibitors were protective in adult brain slices as well. The data suggest that, depending on the age, phenformin and metformin may protect the brain against ischemic damage possibly by suppressing mPTP via inhibition of mitochondrial Complex I.

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47. TRANSCRIPTOME STUDY OF PANCREATIC NEUROENDOCRINE TUMOUR TISSUE

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Introduction: Pancreatic neuroendocrine tumours (PanNETs) arise from the neuroendocrine cells of the pancreas and account for approximately 9% of digestive system NETs. PanNETs are heterogeneous lesions, classified according to cell proliferation rate and differentiation grade, further divided into functional PanNETs due to specific hormone hypersecretion and non-functional PanNETs. In this study, we aimed to create a well-defined cohort of PanNETs and characterize the transcriptomic landscape to discover novel markers for better tumour management options.

Materials and Methods: PanNET FFPE samples used in this study were obtained from different consortium members. RNA was then extracted from FFPE samples and libraries prepared. Transcriptome sequencing was then carried out on DNBSEQ-G400 platform (MGI) to identify differentially expressed genes (DEGs) in tumour and non-tumour tissues.

Results: We have sequenced transcriptomes of 73 PanNET FFPE samples and detected different transcriptome profiles that distinguish tumour and non-tumour tissues of the pancreas. In total, we were able to find over

1000 DEGs. Among these were markers related to tumour pathogenesis pathways, cell proliferation, and invasion.

Conclusions: The results of the PanNET transcriptomic landscape highlight the heterogeneity of PanNETs, which is dependent on various tumour characteristics. Transcriptome analysis is valuable for understanding PanNET tumour biology, which will help to discover new markers and develop new therapeutic approaches for PanNETs.

48. USING DECONVOLUTION TO DISSECT CELL-CLUSTER-SPECIFIC TRANSCRIPTOME PROFILES OF THE HUMAN PREOVULATORY FOLLICLES TO REVEAL GENE EXPRESSION DISTURBANCES BETWEEN HYPO-AND NORMORESPONDERS

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Functional preovulatory follicle cells are critical for human fertility, even though their subpopulations are still unknown. Unfortunately, there are patients, regardless of age, with insufficient responses to follicle-stimulating hormone therapy and therefore have limited oocyte number and pregnancy rate outcomes. This condition affects up to 30% of women undergoing *in vitro* fertilization (IVF) treatment. The mechanism of hyporesponse is not fully explained yet. We aimed to investigate follicular cell clusters of hyporesponders in detail with the use of single-molecule RNA-seq in combination with bulk RNA-seq data and the CIBERSORTx deconvolution group-mode method. We collected preovulatory follicle-derived cells from women undergoing IVF treatment at Nova Vita Clinic, Estonia (10 patients

with normoresponse and 9 patients with hyporesponse). Hyporesponse to treatment was diagnosed if ≥200 IU of rFSH was administered to retrieve an oocyte. All study participants were ≤40 years of age and with normal ovarian reserve. The single-cell transcriptome profile of the preovulatory follicle identified 14 cell clusters. Associating bulk RNA-seq with single-cell RNA-seq results, we distinguished 9 genes specifically highly expressed in progesterone-producing luteinized granulosa cell cluster that were differentially regulated between study groups, mostly downregulated in hyporesponders with statistical significance. Applying deconvolution to study cell-cluster-specific transcriptional differences enabled us to confirm it as a reliable and cost-effective method to impute gene expression from bulk RNA-seq samples.

49. IN SEARCH OF BIOMARKERS FOR TYPE 1 DIABETES

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The composition and function of mononuclear cells are altered under type 1 diabetes (T1D) conditions, in response to individual factors. Based on the methodology described by Grube et al. 2018[1], it is possible to analyse in the peripheral mononuclear cells of T1D patients the intrinsic vibration properties of metabolites, which may serve as a biomarker to monitor the progress of T1D and the development of possible diabetic complications.

Methods: In the study participated 24 T1D patients and 26 individuals without a diagnosis of impaired glucose metabolism. Clinical and anthropometric parameters were determined. Peripheral blood was collected, and peripheral mononuclear cells were isolated and frozen in PBSx1 at -20°C. They were subsequently analysed by FTIR microspectroscopy. Small amount of sample was pipetted onto diamond anvil cell and FTIR spectra registered in range of 4000-600 cm^{-1} as in Grube et al.[1] Statistical analysis was performed using the SPSS Statistics 22.0 program.

Results: There is a shift between absorbance maximum of symmetric PO_2 vibrations in nucleic acids or phospholipids, and C-O stretching in

carbohydrates at 1080-1100 cm^{-1}, indicating on compositional differences between samples. Both Amide I (1656 cm^{-1}) and Amide II (1545 cm^{-1}) peaks show slight shift in their maximum, the latter being statistically significant, thus showing changes in protein content and secondary structure.

Discussion and conclusions: The data obtained indicate very well both the individual differences between patients and the potential of the method to find specific markers of impaired glucose metabolism that could, after further investigation, be used as markers of diabetes progression and identifiers of diabetic complications development.

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50. TOWARDS IMPROVING ADAPTABILITY AND RESILIENCE OF PERENNIAL RYEGRASS FOR SAFE AND SUSTAINABLE FOOD SYSTEMS USING GENOME EDITING – EDITGRASS4FOOD

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Due to an increase in the consumption of food, feed, fuel and to meet global food security needs for the rapidly growing human population, there is a necessity to breed for high yielding crops that can adapt to future climate changes. Perennial ryegrass (*Lolium perenne*) is the dominant forage grass species in Europe due to its high regrowth capacity, rapid establishment, tolerance to frequent cutting and grazing, and high nutritive value for ruminant livestock. However, perennial ryegrass exhibits poor performance under unfavorable environmental conditions compared to other cool

season forage grass species, thus the changing climate pose a substantial challenge to perennial ryegrass cultivation in the Baltic/Nordic region. EditGras4Food project aims at improving perennial ryegrass for winter hardiness, persistence and biomass formation under water-limited conditions. This will enable us to utilize the gained information in future genomic selection programs to develop ryegrass cultivars with improved freezing and drought tolerance and persistence. It will also help breeders and agriculture in general in the Nordic/Baltic region to prepare for meeting new demands due to climate change and changing societal demands. Importantly, by improving forage production, dairy and meat industries will directly benefit and therefore this project contributes to safe and sustainable food systems. EditGrass4Food project utilizes unique pre-breeding material, developed by the Nordic/Baltic Public-Private Partnership project, as well as CRISPR-based editing to validate candidate genes involved in northern adaptation of this crop. In this study, we focus on bioinformatic identification of genes involved in the mechanisms of freezing tolerance and biomass growth under water deficit. Briefly, we use known abiotic stress-related gene sequences from different grass species to identify homologous sequences in perennial ryegrass draft genome sequence (https://ryegrassgenome.ghpc.au.dk/) or in *Lolium rigidum* genome sequence (https://www.ncbi.nlm.nih.gov/genome/?term=txid89674[Organism:noexp]) and to annotate intron-exon structure for identification of target regions for genome editing.

51. OPTIMIZATION OF CRYSTALLIZATION CONDITIONS FOR STABILIZED TUBULIN COMPLEX

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Microtubules are one of the most essential components of the cytoskeleton in eukaryotic cells, which are involved in various basic cell functions, as mitosis, morphogenesis, proper division of the chromosomes, etc. Due to involvement in cellular key functions, microtubules are extremely important

targets for cancer and neurological disorder drugs also known as microtubule-targeting agents[1]. Microtubules are dynamic protein filaments made up of α, β-tubulin heterodimers[2,3].

In order to perform structural studies on tubulin binding sites, it is crystallized in form of stabilized complex with stathmin-like protein RB3 and tubulin-tyrosine ligase (TTL)[4]. Here, we adjusted existing crystallization protocols to find optimal conditions suitable for fragment screening by X-ray crystallography. Tubulin was the product of Cytoskeleton Inc. (Cat. # T240) while RB3 and TTL were expressed in place. RB3 was produced according to previously published protocol[5]. However, TTL was found to be extremely instable while expressed in E. Coli. Thus, we developed new protocol for expression and purification of TTL as a fusion with maltose binding protein (MBP). Various ratios of apo tubulin, RB3, and TTL were tested by size exclusion chromatography (Superdex200*pg* 10/300 Increase).

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52. PRO-INFLAMMATORY S100A9 PROTEIN EFFECT ON TAU PROTEIN AGGREGATION

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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 [1]. Alzheimer's disease has attracted the most attention from scientists because it 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 [2]. Therefore, it is essential to understand the mechanisms at work in this process and determine the best way to curb them. Amyloid-β aggregates (and α-synuclein aggregates in Parkinson's disease) have been shown to promote Tau aggregation [3]. It has also been observed that the aggregation of these two peptides involves the pro-inflammatory protein S100A9, whose elevated levels in the brain are recorded after various head injuries.

Furthermore, one other tauopathy – CTE (Chronic traumatic encephalopathy) – registers high levels of Tau aggregates, and the exact reasons for their formation are unknown. Researchers observed that this disease is quite prominent in contact sport players (e.g., American football) who experiences chronic head concussions [4]. 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 [5]. 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.

In some experiments, polyanion heparin was used as an initiator of amyloid protein aggregation *in vitro* to determine the optimal aggregation conditions for Tau protein prior to experiments with S100A9 protein. Aggregation kinetics were recorded by fluorescence spectroscopy using the amyloidophilic dye thioflavin T. Atomic force microscopy was performed to analyze the morphology of the formed aggregates.

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53. EFFECT OF VEGAN DIET ON EXOGENIC LIPOPROTEIN LIPASE ACTIVITY IN HEALTHY INDIVIDUALS

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Lipoprotein lipase (LPL) is a key enzyme involved in fatty acid partitioning and a potential drug target for the treatment of hypertriglyceridemia. LPL activity is regulated post-translationally by multiple activators and inhibitors in a tissue-specific manner depending on the nutritional status of the organism. Several studies have shown that post-heparin LPL levels and activity can be affected by the fatty acid and micronutrient composition of the diet. Previous works have also investigated the effect of a vegan diet on lipid metabolism and related lipoprotein blood markers but so far, conclusions have not been made on its effect on LPL activity.

In this study, isothermal-titration calorimetry was used to compare the activity of exogenic LPL in fasted blood serum obtained from vegans or omnivores. Participants were asked to complete a seven-day food diary, which was then used to summarize the fatty acid composition of the diets. Preliminary data showed no statistical difference in exogenic LPL activity between the two diets but interestingly, results revealed individuals in both diets with highly similar levels of triglycerides whose LPL activity varied more than two-fold. Further research is required to elucidate the effect of specific nutrients on LPL activity.

54. DEVELOPMENT OF PROTEIN KINASE INHIBITORS THAT LOSE AFFINITY UPON PHYSICAL OR CHEMICAL ACTION

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Compounds that concomitantly associate with two distinct binding sites of the same protein provide higher affinity if compared to compounds that only bind to single site. Such compounds – e.g., bitopic ligands and bisubstrate inhibitors – are conjugates of two fragments, each fragment targeted to one specific binding site. The free energy change of the binding of such conjugates to their targets is expressed as the sum of three components. Two of these components correspond to the binding energies of the aforementioned fragments and the third summarizes all other factors including entropic benefit, which arises from the conjugation of the fragments (avidity or chelate effect).

Previously, we have developed bisubstrate inhibitors called ARCs that tightly bind to the active centre of a target protein kinase (PK).[1] ARCs are conjugates of two fragments that concomitantly occupy the ATP-binding pocket and the protein substrate-binding region of the catalytic domain of a PK. These fragments are joined via a linker. Recently, we showed that when the binding of the fragment targeted to the ATP-binding pocket was blocked by a photocage, the affinity of the conjugate was reduced by up to 5 orders of magnitude.[2]

In the present work, we constructed ARCs in which the fragments of the conjugates were connected via destructible linker. Two types of linker were used: one containing a photodegradable moiety, the other disulphide bond. Depending on the type of the linker, irradiation or chemical reduction of the complex of the ARC with the catalytic subunit of cAMP-dependent PK decomposed the ARC into low-affinity fragments that resulted in the liberation of the free catalytic subunit of the PK.

The incorporation of destructible linker into conjugates such as ARCs opens new possibilities for developing compounds and materials that temporarily inhibit or capture enzymes. The findings of this and the previous2) work demonstrate that the bisubstrate inhibitor design offers great flexibility for constructing inhibitors that can be activated and/or deactivated by external stimuli.

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55. THE EFFECT OF *ELSHOLTZIA CILIATA* ESSENTIAL OIL ON THE OXIDATIVE STRESS IN MICE ORGANS

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Background: Recently data show that *Elsholtzia ciliata* (*E. ciliata*) essential oil has an antioxidant activity which is determined by different phenolic compounds. Flavonoids are the most predominant substances in the genus *Elsholtzia* and have an effect against oxidative stress which plays an important role in the development of various diseases. **The present study aimed** to determine the effect of *E. ciliata* essential oil on biomarkers of oxidative stress in blood and heart of laboratory mice.

Materials and methods: The experiment was done on 4–6 weeks old white BALB/c laboratory mice weighing 20–30 g. 50 mice were divided into five groups: control, which received saline solution; olive oil; $AlCl_3$; *E. ciliata* oil and $AlCl_3$; *E. ciliata* essential oil. Oxidative stress was induced by injection of $AlCl_3$ solution. The concentrations of reduced glutathione (GSH), malondialdehyde (MDA) and the activity of catalase (CAT) in blood and heart of mice were assessed by spectrophotometric analysis after 21 days. The difference was considered statistically significant at value of $p < 0.05$.

Results: *GSH levels in blood of mice treated only with essential oil significantly increased by 28.1% ($p < 0.05$) compared to control group. Essential oil significantly reduced the concentration of MDA in blood of mice exposed to $AlCl_3$ by 40.8% compared with control group. E. ciliata L. essential oil significantly reduced MDA level in heart by 22.7% in the*

presence of AlCl3 and remained similar with essential oil administration alone (23.2%) compared with control group ($p < 0.05$). After $AlCl_3$ injection administered with essential oil significantly increased CAT activity by 30.1% compared with $AlCl_3$ group and by 15.1% in heart of mice treated only with essential oil compared with control group ($p < 0.05$).

Conclusions: *E. ciliata* L. essential oil reduced the concentration of lipid peroxidation product MDA, restored decreased GSH concentration and CAT activity after $AlCl_3$ induced oxidative stress, and activated these natural antioxidants in blood and heart of mice treated only with essential oil. Results confirm the antioxidant properties of the *Elsholtzia ciliata* L. herb.

Keywords: *Elsholtzia ciliata*, oxidative stress, mice

56. INTERACTION AND DYNAMICS OF INTESTINAL IGA AND GUT MICROBIOME PROFILE DURING ANTIDIABETIC THERAPY

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Background: Metformin is one of the most commonly used drugs for the treatment of type 2 diabetes. Recent studies suggest that metformin significantly alters intestinal microbiome in patients with T2D and scientists have identified the gut as a potential target of metformin. The secretory immunoglobulin A (sIgA) plays an important role in the protection and homeostatic regulation of the intestinal, respiratory, and urogenital mucosal epithelium, which separates the external environment from the inside of the body. Its interaction with gut microbiome among individuals and populations varies widely, but the factors behind these differences are still unclear. Recent studies have shown that metformin therapy could be associated with changes in sIgA levels in the gut, therefore, indicating a possibly new mechanism of action for the observed microbiome changes and microbiome mediated therapeutic effects.

Aims and method: The overall aim of the study is to understand the modulatory effect of sIgA on gut microbiome in T2D individuals. Stool samples from T2D patients were collected at three consecutive time points: (1) before metformin therapy, (2) after one-week metformin

therapy, (3) after three months of metformin therapy. Stool samples were sorted using magnetic beads-based cell separation method. For each sample, the following two sample types were obtained and further analyzed: presort and sIgA+ fraction. From both fractions microbial DNA was extracted using the MGISP-100 Automated Sample Preparation System and MagPure Stool DNA LQ Kit and were characterized using shotgun metagenomic sequencing.

Results: We compared the taxonomic composition both longitudinally and cross-sectionally across all sample types at each of the analyzed time points. We determined the most characteristic taxonomic groups within each of the fractions and we observed metformin induced increase and reduction in abundance of several species specifically in the sIgA+ fraction. Metformin administration in T2D cohort induced decrease in abundance of *Alistipes*, *Rikenellaceae* (Presort sample), *Proteobacteria* (IgA+ sample) and increase of *Roseburia* (Presort + sample).

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57. THE EFFECTS OF IMEGLIMIN ON DEVELOPING BRAIN MICROGLIAL CELLS AFTER HYPOXIA AND REOXYGENATION

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Brain ischemia induces cell death via multiple mechanisms in which microglia plays an important role. However, there is still lack of knowledge about the mechanisms and consequences of microglial activation during ischemia. There are some data on neuroprotective effect of antidiabetic drugs after ischemic brain injury though the way of action on microglia activation has not been investigated yet. The aim of this study was to investigate the role of novel antidiabetic drug – imeglimin – on primary rat microglial cell cultures in hypoxia/reoxygenation conditions. Microglial viability, proliferation, phagocytic, metabolic activities and RNS changes in culture after 24 hours of hypoxia (2% O_2) and 24–120 hours of reoxygenation in the absence/presence of imeglimin were evaluated.

Our results showed that in pure microglial cell cultures, neither hypoxia nor prolonged reoxygenation, as well as imeglimin had no effects on viability and proliferation. We also determined that phagocytic activity of the microglial cells after 24h hypoxia and 24h reoxygenation was increased whereas imeglimin had an activity-reducing effect. Further we evaluated the changes of cellular RNS production after hypoxia and reoxygenation – NO level was increased only after prolonged reoxygenation (120h) as pre-treatment with imeglimin partly reduced NO production.

These data suggest that hypoxia tent to stimulate microglial activation and imeglimin may have activation reducing effect.

58. ARE THE COURSE AND SEVERITY OF COVID-19 RELATED TO SELENIUM, SELENOPROTEIN P AND BLOOD OXIDATIVE STRESS LEVEL IN PATIENTS WITH ACUTE AND post-COVID-19 DISEASE?

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Hypothesis: Whether selenium (Se), selenoprotein P (SelP), and oxidative stress (OS) affect the course and severity of COVID-19 disease, and whether post-infection effects may depend on a variety of factors, including Se, SelP, and OS levels in the body.

Materials and methods: Study was performed in 120 patients, including 40 in the acute phase of the disease and 80 patients after COVID-19. Se content was determined by a fluorometric method with diaminonaphthalene using acidic hydrolysis (Alfthan G. et al.), SelP, MDA and 4-HNE adducts and their metabolite adducts were evaluated by spectrophotometric methods using commercial assay kits.

Results and conclusions: According to the results, we can conclude: in case of severe illness, the patient has lower (deficient) concentrations of Se and SelP. Therefore, in case of OS, the opposite tendency is observed – the more severe the course of the disease, the higher the level of OS, which argues significantly more intensive formation of oxygen free radicals in the body. We recommend to use organic Se preparations and natural antioxidants as adjunctive therapy for disease prevention and recovery.

59. INSIGHTS OF SCV-LBC GAG EXPRESSION IN *SACCHAROMYCES CEREVISIAE* STRAINS

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Yeast *Saccharomyces cerevisiae* is one of the best understood eukaryotic model organisms. It is used for research of various biological processes like gene expression, cell cycle, and metabolism, as well as for virus research[1,2]. ScV-LBC is an endogenous 4,6 kb dsRNA yeast virus belonging to *Totiviridae* family. ScV-LBC codes two ORFs: a major cap protein Gag and an RNA dependent RNA polymerase Pol, expressed as a Gag-Pol fusion protein. The latter is expressed only when a ribosomal frameshift event occurs, controlling the ratio of the two proteins, needed for viral replication[3,4]. Here we investigate the patterns of LBC Gag protein expression and localization in *S. cerevisiae*.

In this work, we fused LBC Gag protein to mCherry fluorescent protein either in the same frame or with a frameshift signal in between. The fusion protein was expressed in several *S. cerevisiae* strains that differ in genetic environment and/or endogenous viruses possessed. Fluorescence patterns in unstained SDS-PAGE mode and in yeast cells observed using fluorescent microscopy varied depending on the strains and the type of LBC-mCherry construct. Finally, bioinformatic analysis is in progress to identify the genetic differences of the strains behind the cause of different LBC Gag biosynthesis efficacy in different *S. cerevisiae* strains.

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60. CHANGES OF IMMUNE RESPONSE RELATED GENES EXPRESSION PROFILE IN ENDOMETRIUM TISSUE OF FEMALES WITH REPRODUCTIVE DISORDERS

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Infertility affects up to 9–18% of the total population[1]. Successful pregnancy depends on the susceptibility of endometrial tissue and the interaction between the endometrium and the blastocyst during embryo implantation. Altered embryo-endometrial dialogue is estimated to be responsible for two-thirds of implantation failures[2]. Immunological imbalance in cells in reproductive tissues can lead to negative outcomes of pregnancy, such as recurrent spontaneous abortion, preeclampsia, preterm birth, infections, intrauterine growth restriction, etc.[3].

In this research we examined endometrial tissue samples from 11 reproductive healthy patients and 14 patients diagnosed with various reproductive disorders, including unexplained infertility, endometriosis, and Fallopian tube pathology. The expression of genes related to immune response was examined by using RT-qPCR. The analysis of the expression of genetic markers of immune response and inflammation in endometrial tissue showed that expression of *NFKB1*, *NFKB2*, *REL* and *RELA* is increased in endometrium of patients with reproductive disorders. Genes coding interleukins (*IL1B*, *IL2*, *IL18*), *INFG*, *CXCL1* and *CXCL10* are upregulated in endometrium of patients with reproductive disorders compared to reproductive healthy patients.

We suggest that increase of immune response related gene expression in endometrium of patients with diagnosed reproductive disorders could indicate state of inflammation and may have impact on reproductive function.

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61. ENGINEERING OF CHIMERIC CARBONIC CNHYDRASES FOR DESIGNING ISOFORM-SELECTIVE INHIBITORS

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A family of human carbonic anhydrases (CA) contains 12 catalytically active isoforms that play a role in various diseases, including cancer, glaucoma, epilepsy, and altitude sickness. Unfortunately, CA inhibitors used as drugs lack selectivity for the target isoform and cause serious side effects. In addition, because the 12 CA isoforms have a high percentage of amino acid sequence homology, it is challenging to design isoform-selective inhibitors.

To understand the mechanism of inhibitor recognition and selectivity profiles, we have generated chimeric carbonic anhydrases that resemble CA Va, CA VI, or CA XII. The off-target isoform – CA II was used as a core, and 5-6 amino acids in the active site were mutated to those specific for CA Va, CA VI, or CA XII. The binding affinities of a series of benzenesulfonamides binding to the off-target, chimeric, and target isoforms were determined using isothermal titration calorimetry, enzymatic inhibition, and thermal shift assays. In addition, X-ray crystallography and computational modeling were used to compare the binding modes of selected inhibitors in the active sites of native and engineered CA isoforms.

The detailed thermodynamic and structural analysis revealed that chimeric carbonic anhydrases recognized and bound inhibitors with similar binding affinities and binding modes as target isoforms (CA Va, CA VI, and CA XII), but not as the off-target isoform. Thus, the chimeric carbonic anhydrases were confirmed as valuable models for designing isoform-selective inhibitors. The general strategy could be applied to other drug discovery projects.

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62. ANTAGONISTIC TRAITS OF YEASTS ISOLATED FROM THE SURFACE OF SOUR AND SWEET CHERRIES

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Sour cherries (*Prunus cerasus* L.) and sweet cherries (*P. avium* L.) are widely cultivated and consumed berries. Cherry fruits are nutrient-dense and have considerable amounts of bioactive components, promoting human health. Fruits tend to have higher sugar content than the rest of the plants, thus berries are attractive hosts of various microorganisms, including yeasts.

The aim of the current study was to isolate and identify the cultivable yeasts associated with sweet and sour cherries to explore their biocontrol potential. Yeast cultures were isolated by culture-dependent approaches and identified by molecular methods; their antagonistic activity was assessed against other yeasts, including potential pathogens.

We analysed cultivable yeasts associated with the carposphere of sour and sweet cherries that were freshly harvested from private plantations and purchased in a food store. In the present study, different yeast strains from *Aureobasidium*, *Metschnikowia*, *Hanseniaspora*, *Saccharomyces*,

Pichia, and *Torulaspora* genera were isolated. They possess a broad spectrum of antagonistic activity against other yeasts, including potential pathogens. The prominent biocontrol activity of cultivable yeasts isolated from freshly harvested cherries against potentially pathogenic microorganisms highlights prospects of these strains in the management of disease control. This research employs natural resources to explore the desirable properties of environmental yeasts, with particular interest in development of strategies effective in the fight against potential pathogens. This research was funded by European Social Fund (09.3.3-LMT-K-712-01-0099) under grant agreement with the Research Council of Lithuania (LMTLT).

63. AMINOTRANSFERASES FOR CHIRAL AMINE SYNTHESIS

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Aminotransferases (AT) are enzymes that mediate the transfer of an amino group from an amino donor to a ketone acceptor. The catalysis by ATs is performed under mild conditions, without the use of toxic metals and solvents, and has a higher stereo- and regio-selectivity compared to the organic synthesis [1].

In this study, we searched for ATs in the metagenomic DNA libraries using indol-3-ylmethylamine. We found and recombinantly expressed 18 different ATs. ATs were active towards a wide variety of aromatic and aliphatic keto compounds. In small-scale amination experiments, we were able to achieve a conversion of 95% for 2-acetylpyridine and 2-indanone. Furthermore, some ATs showed activity towards monosaccharides and, therefore, could be employed for the synthesis of different aminopolyols – a class of products that are of particular interest as carbohydrate mimetics.

In summary, we successfully identified ATs active towards a wide scope of keto compounds.

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64. ISCHEMIA *IN VIVO* CAUSES CARDIOLIPIN PEROXIDATION AND MITOCHONDRIAL DAMAGE IN RAT KIDNEYS

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Cardiolipin is a mitochondrial phospholipid that is mainly found in the inner membrane. Cardiolipin interacts with mitochondrial proteins and stabilizes OXPHOS complexes, cristae structure, participates in mitophagy and apoptosis. Cardiolipin is prone to peroxidation due to its location near electron transport chain and because cardiolipin contains four polyunsaturated fatty acids. Therefore, mitochondrial reactive oxygen species (ROS) formation may cause damage to cardiolipin which can further impair mitochondrial function. Kidney ischemia/reperfusion injury occurs during kidney diseases, surgeries (e.g., transplantation, tumorectomy), and is characterized by oxidative stress and elevation of ROS. However, ROS are generated even during ischemia and can induce cardiolipin peroxidation with further mitochondrial damage. We have induced kidney ischemia *in vivo* in adult male Wistar rats for 30, 40 and 60 minutes, analyzed kidney mitochondrial respiration and structural alterations of mitochondrial cardiolipin. It was observed that already after 30 minutes of *in vivo* ischemia mitochondrial respiration in the presence of glutamate/malate and ADP decreased about 37 %, and further decreased 65 % and 73 % after 40 and 60 minutes of ischemia respectively. Along impaired mitochondrial function we observed an increase in cardiolipin peroxidation – 40 minutes of *in vivo* ischemia caused an average 6,9-fold increase of eight peroxidized tetralinoleoyl cardiolipin

species with additional one to eight oxygen atoms. These cardiolipin species contained linoleic acids with up to four oxygen atoms yielding linoleic acid hydroxides, hydroperoxides and dihydroperoxides.

65. MUA-BASED MOLECULAR INDEXING FOR RARE MUTATION DETECTION BY NEXT-GENERATION SEQUENCING

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Detection of low-frequency mutations in cancer genomes or other heterogeneous cell populations requires high-fidelity sequencing. Molecular barcoding is one of the key technologies that allow to differentiate between true mutations and errors resulting from sequencing or library preparation processes. However, current approaches of introduction of barcodes via primer extension or adaptor ligation do not utilize the full barcoding power due to complicated library preparation workflows and biases. Here we demonstrate the remarkable tolerance of MuA transposase to the presence of multiple replacements in transposon sequence, and explore this unique feature to engineer the MuA transposome complex with randomised nucleotides in 12 transposon positions, which can be introduced as a barcode into the target molecule after transposition event. We applied the approach of Unique MuA-based Molecular Indexing (UMAMI) to assess the power of rare mutation detection by shortgun sequencing on Illumina platform. Our results show that UMAMI allows detection of rare mutations readily and reliably, and report error rate values for the number of thermophilic DNA polymerases measured by using UMAMI.

66. APPLICATIONS OF NEW FLUORESCENT DISUBSTITUTED BENZANTHRONE DYES FOR DNA STUDY

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Cell molecular biology is very rapidly developing through both traditional and modern fluorescent assay methods. The importance is the detection of fluorescent dyes, which is specific for the staining of cellular structures and for the visualization of nucleic acids, proteins, and quantitative determination of its. Previous studies have shown great promise for using new fluorescent disubstituted benzanthron dyes as to analyze intracellular structures and molecules.

The present research is aimed at the application of new fluorescent disubstituted benzanthrone dyes, as in molecular and cell biology.

26 fish and wheat DNA samples were obtained by the salt-out method and were examined for contamination (the ratio of absorbance at A260/280nm and A230/260nm was used to assess the purity of DNA from protein, EDTA, carbohydrates, phenol, or other contaminants) and DNA concentration by spectrophotometric analysis and DNA fragmentation by gel-electrophoresis.

The different spectrums of absorption and fluorescence of DNA with new fluorescent disubstituted benzanthrone dyes were obtained and analyzed. The use of new fluorescent disubstituted benzanthrone dyes for DNA analysis and staining of intracellular structures was analyzed.

67. EFFECT OF PRO-ANTIARRHYTHMIC *ELHOLTZIA CILIATA* ESSENTIAL OIL ON BLOOD PARAMETERS IN SWINE

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Introduction: In Western society, the cardiovascular disease is increasing every year, the need for the development of new pharmaceuticals is growing. The newly discovered substances appear to have many side effects and even cause mortality [1]. Herbal extracts are becoming very popular and it is estimated that countries e.g. Germany, France never abandoned herbal medicine [2]. Interest in medicinal plants that have antibacterial properties is also growing strongly [3], therefore scientific and business companies are greatly interested in the use of it [4]. An annual plant *Elsholtzia ciliata* (EC) essential oil possess antiarrhythmic [5], antiviral [6] and antibacterial [7] properties.

Aim: Evaluate the effect of *Elsholtzia ciliata* essential oil on swine blood parameters.

Methods: eight Lithuanian local breed pigs weighted 32 ± 1.9 kg were used in following experiments, during procedure animals were unconscious under general anaesthesia. All experiments were performed according to the European Community guiding principles and permitted by responsible authorities. The test substance using same dose 30 µg/kg was injected into ear vein. Blood samples for blood haematology and blood gas and biochemistry analysis, were taken from common jugular in this order: T0 – control value, T1, T5, T30 – number indicates minutes after injection.

Results: The hematological parameters of the control and other time points after *E. ciliata* essential oil were analysed. The WBC decreased significantly ($p < 0.05$) one minute after injection, followed by growth, at T30 WBC count was insignificantly lower than the control measurement. The significant decrease in PLT also observed at the T1 time point, at T30 measures were cloe to control values. After EC injection potassium increases

insignificantly at T1, therefore its value decrease significantly ($p < 0.05$) at T5 ant T30. Other blood mineral parameters changes insignificantly after EC injection.

It can be concluded that there is no significant change in the swine blood composition after EC injection.

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68. DIFFERENT INDUCERS IMPACT FOR ENDOMETRIAL STROMAL CELLS IN VITRO DECIDUALIZATION

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Infertility affects 15 % of reproductive-aged couples worldwide. Each menstrual cycle endometrium undergoes massive changes, including tissue regeneration, proliferation, and differentiation. The efficiency of endometrial stromal cells (ESC) decidualization is the critical player in successful embryo implantation and further pregnancy development. The accurate *in vitro* ESC decidualization model is needed to investigate

the endometrial cycle's molecular regulation and could help identify endometrial targets for infertility treatment solutions. In this study, ESCs were isolated from an endometrial biopsy of patients preceding assisted reproductive technology. Isolated ESCs were characterized by evaluating surface markers and differentiation potency. It has showned that ESCs express mesenchymal (CD90, CD73) and endometrial stem cell (CD146, SUSD2) surface markers and have a broader than endometrial tissue-specific differentiation potential. Further more, ESCs were induced to differentiate in decidualization specific manner, using different inducers (10 nM estradiol (E2), 1 μM progesterone (P), 0.5 mM cAMP (or its derivatives, db-cAMP, 8 br-cAMP), and 1 μM medroxyprogesterone acetate (MPA)). Decidualization efficiency using different inducers was compared morphologically and quantitively by measuring decidualization markers (PRL, IGFBP-1) gene expression changes after six days of *in vitro* decidualization. Results revealed that E2, MPA, or P are weak inducers of decidualization markers expression however, cAMP stable derivatives db-cAMP and 8 br-cAMP lead significantly *PRL* and *IGFBP1* expression increase during induced ESC in vitro decidualization. Moreover, *PRL* and *IGFBP1* expression were even higher, then in combination with stable derivatives of cAMP, MPA was added. Induction media consisting of 0.5 mM db-cAMP and 1 μM MPA was selected as the most suitable for further *in vitro* ESC decidualization study.

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69. EFFECT OF CELL CULTURE CONDITIONS ON CELLULAR BIOENERGETICS AND VIABILITY

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Cell models play a central role in preclinical research aimed at the mechanism of disease and drug discovery[1]. Alterations in cell antioxidant response and bioenergetics profile are at the root of many diseases. Thus,

the *in vitro* model of disease must also faithfully reproduce these disturbances. The activity of bioenergetic pathways and antioxidant response is regulated by the outside environment of the cells, including levels of nutrients and oxygen tension. At the same time, the composition of commonly used cell media deviates significantly from the composition of interstitial fluid in our body[2]. This study aimed to evaluate the effect of substitution of classic cell media (DMEM) with cell media in which the composition of nutrients matches human plasma (Plasmax) on cell viability and respiration. Results show that rat neuroblastoma B35 cells grown in physiological media show a lower activity of respiratory complexes and increased sensitivity to toxic compounds as compared to cells grown in regular cell culture media. For reasons of convenience, traditional cell culture work is done at ambient oxygen pressure (21 kPa) while partial pressure of oxygen is just 4-5 kPa in most tissues[3]. Metabolic profiles of astrocytic and neuronal cell lines showed different sensitivities in response to varying oxygen tension. These results illustrate the importance of finding proper conditions on cell growth and the effect it has on cell viability, antioxidant response, and mitochondrial respiration.

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70. YEAST-BASED SYSTEM FOR *IN VIVO* EVALUATION OF ALLELES OF THE ANTHOCYANIN PRODUCTION PATHWAY

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Plants produce anthocyanins to incite the pollination and seed dispersion performed by pigment-attracted animals. These natural blue-to-red-coloured pigments can be used as food colorants and antioxidants. For this

purpose, microbial bioproduction of anthocyanins has become of industrial interest in recent years[1]. The anthocyanin biosynthetic pathway in plants is composed of 7 genes that transform L-phenylalanine into anthocyanidins. Additionally, red-to-blue colour shift is enhanced by flavonoid 3'5' hydroxylase (F3'5'H), and stable anthocyanins are achieved after glycosylation by UF3GT. In this study, the episomal complementation system to evaluate enzyme variants was created and tested in the yeast *S. cerevisiae*. The allele of interest was introduced in 2μ plasmid form to the strains to complement the pathway. The pathway was divided into two sections. The first section is composed of 4CL2, CHS, and CHI to produce naringenin from media-supplemented p-coumaric acid. The second section contains F3H, DFR, and ANS genes to produce pelargonidin from supplemental or produced naringenin. This section can be episomally supplemented with alleles of F3'5'H and/or UF3GT. To evaluate the enzyme activity, methanol extracts from the yeast cultures are analysed by the LC-MS system to quantify the products and compare them to those produced by the base strain. All enzymes except F3'5'H are synthesised in yeast as soluble N'-terminal polyhistidine tagged proteins. F3'5'H is produced as a C'-terminal tagged, membrane-anchored protein. This system has been employed to evaluate the *in vivo* activity of 24 alleles. The episomal complementation approach is flexible for further optimisation for high throughput screening applications.

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71. ORGANOSOLV LIGNIN DEGRADATION BY *ACTINOMYCETALES*

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Lignocellulosic biomass (LCB) is the most abundant renewable raw material in nature, and the main constituent of plant cell walls composed of cellulose, hemicellulose, and an aromatic biopolymer lignin. Both the covalent and non-covalent bonds strongly interlink together these polymers, making assembled structure recalcitrant to enzymatic conversion.

Agricultural, forest, and industrial activities generate tons of LCB wastes annually and therefore the focus to develop new technologies and materials has been based on LCB as a raw material for the last decade.

In nature, degradation of LCB is performed by microbial consortia that synthesize a broad range of hydrolytic and non-hydrolytic enzymes with different properties. Lignin is one of the most reluctant components of LCB and fungi are considered as the best lignin degraders. Despite that, the lignolytic bacteria are more adaptable than fungi, and their enzymes work optimally at wide range of temperature and pH. Gram-positive bacteria belonging to order *Actinomycetales* found both in terrestrial and aquatic habitats are major degraders of LCB and produce a repertoire of redox-active lignin degrading enzymes.

We are testing the ability of different *Actinomycetales* species to utilise lignins extracted by organosolv method from aspen, pine and barley biomasses. We perform lignin degradation flask experiments for two weeks to examine the potential of different bacterial species to utilize different lignins as sole carbon sources. Our ultimate goal will be to decipher the cocktail of lignin degrading enzymes as well as characterize the lignin depolymerization products. Size exclusion chromatography and GC-MS are performed to evaluate the lignin degradation rate and to determine specific degradation products. The cocktail of lignolytic enzymes secreted to the growth medium will be identified using proteomic methods such as comparative secretome analysis.

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72. DEVELOPMENT OF VISIBLE-LIGHT ACTIVATED PHOTOCAGED BISUBSTRATE INHIBITOR OF PROTEIN KINASES

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Photocaging – temporal deactivation of compounds by derivatization with photolabile groups – is an emerging approach in the development of biochemical tools and pharmacological agents for targeting biochemical pathways in fine spatiotemporal resolution. Illumination of the photocaged compound at the right wavelength induces a photochemical reaction, which irreversibly cleaves the photocage and liberates the bioactive compound.

Previously, we have developed bisubstrate inhibitors called ARCs that tightly bind to the active centres of protein kinases (PKs).[1] ARCs contain two structural fragments that simultaneously occupy distinct binding sites on the same protein – the ATP-binding pocket and the protein substrate-binding region of the catalytic subunit of a PK. Recently, we demonstrated that an attachment of a nitrodibenzofuran-based photocage to only one fragment of the structure of ARCs fully abolished its binding affinity.[2]

In the present work, a diethylaminocoumarine (DEAC)-photocaged bisubstrate inhibitor ARC-2145 was developed. ARC-2145 was synthesized by solution phase and Fmoc solid-phase peptide synthesis methods. The structures of the intermediates and the final compound were verified by combination of NMR and UV-Vis spectroscopy and mass spectrometry. Competitive displacement assay revealed that ARC-2145 only weakly associated with the catalytic subunit of cAMP-dependent protein kinase (IC_{50} = 26 μM). Irradiation of the photocaged inhibitor resulted in remarkable (3 orders of magnitude) increase of binding affinity (IC_{50} = 38 nM). If compared to the previously published photocaged ARC, DEAC-photocaged inhibitor could be activated by irradiation at longer wavelength (up to 430 nm). This is advantageous in biological systems, because visible light is less hazardous than near-UV radiation used for the cleavage of the nitrodibenzofuran-based photocage.

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73. DISCOVERY OF STRONG INHIBITORY PROPERTIES OF A MONOCLONAL ANTIBODY OF PROTEIN KINASE A

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In this century protein kinases (PKs) have been the most important targets for development of cancer drugs. Already more than 70 FDA-approved medicines are in use in clinic for regulation of aberrant activity of PKs. All these inhibitors bind to the structurally conserved for 538 human PKs binding sites of the co-substrate ATP. Therefore, none of the PK-inhibitory small-molecule drugs is specific for a single PK; that is a cause of possible severe toxicity of the drugs.

Alternatively, several monoclonal antibodies are specific binders for target PKs. Often binding of an Ab is not competitive with binding of the substrates. These antibodies are not inhibitory for the PK-catalyzed protein phosphorylation reaction.

Here we used PK-binding responsive long lifetime photoluminescent probes, developed by us, to fish out a commercial monoclonal Ab (mAb(D38C6)), whose binding to the catalytic subunit α of protein kinase A (PKAcα) inhibits the protein phosphorylation reaction with low-nanomolar inhibitory potency (K_i = 2.4 nM)[1].

The discovered competitiveness between two structurally and mechanistically different binders was used for the construction of an assay for the analysis of PKAcα. The enzyme was specifically captured to mAb(D38C6)-immobilized immunocapture surface and thereafter the active protein was eluted with an orthosteric high-affinity ARC-Lum(Fluo) probe. The novel assay possesses specificity and high sensitivity. The developed assay is low-cost, timesaving, and allows the determination of PKAcα with 50 pM (2.3 fmol) limit of quantification.

In several PKs of the AGC group, Phe residue of the AST region of the C-terminal extension participates in the formation of active core and binding of ATP, therefore it is likely that Ab-s generated against a peptide antigen around this Phe residue would possess inhibitory properties and could be applicable as specific research tools.

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74. METAGENOMIC CHARACTERIZATION OF UNTREATED WASTEWATER ACQUIRED FROM 15 MUNICIPALITIES OF LATVIA

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Untreated wastewater is a chemically and biologically complex environment, but its microbiological composition so far has been little studied. Currently available sequencing and bioinformatics technologies have allowed the characterization of wastewater microorganism communities at different levels. The aim of the study was to taxonomically and functionally characterize the wastewater metagenome of 15 Latvian municipalities. The whole metagenome sequencing method was used to analyse 24-hour composite samples of untreated wastewater that were collected at inlet of wastewater treatment plants. We found that microbiome of analysed wastewater samples is dominated by the *Arcobacter cryaerophilus* (on average 28% of classified sequences on species level), but there were also other common microbial species – *Pseudoarcobacter articola*, *Bacteroides vulgatus*, *Aeromonas media*, *Faecalibacterium prausnitzii*, *Lactococcus rafinolactis*, *Moraxella osloensis*, *Sulfurospirillum cavolei*, *Lactobacillus amylovorus*, *Desulfobacter postgatei*, and *Streptococcus suis*. Acquired data showed that there are statistically significant taxonomic and functional differences in the composition of the metagenome between cities ($p<0.05$) and different inflows of industrial water ($p<0.05$), but some similarities in species, genus, phylum, and gene levels were also observed. Overall, our data showed that the taxonomical and functional composition of wastewater of each municipality was stable over a three-week period and microbial variability between municipalities can be explained by differences in wastewater inflow compositions.

75. PROGNOSTIC INSIGHTS FROM THE ANALYSIS OF UTERINE LAVAGE SAMPLES IN OVARIAN CANCER PATIENTS

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Ovarian cancer is the third most prevalent and second deadliest gynaecologic malignancy in the world[1]. Currently, there is no suitable prognostic and diagnostic biomarkers for ovarian cancer. Uterine lavage has been recently proposed as a novel liquid biopsy technique for improved mutation detection for gynaecologic disease detection and prognostics[2].

The aim of the study was to analyse mutations in tumour and uterine lavage samples in order to determine suitable diagnostic and prognostic biomarkers for ovarian cancer.

Methods: The study included 78 patients (40 high-grade serous ovarian cancer (HGSOC), 8 other ovarian malignancies, 12 endometrial cancer and 18 benign gynaecologic tumours). In all, 75 uterine lavage and 47 tissue samples were analysed for mutations in 10 genes commonly associated with gynaecologic diseases using custom Ion Torrent AmpliSeq On-Demand Panel.

Results: 80% (32/40) HGSOC patients had mutations in *TP53* and *BRCA1/2* genes, meanwhile 35% (7/20) of non-HGSOC patients harboured multiple PI3K and WNT pathway gene alterations. *ARID1A* was the most universally mutated gene, with mutations detectable in 50% non-HGSOC and 15% (6/40) HGSOC patients. 66% (10/15) cases with early disease progression had multiple mutations. Mutation concordance in paired uterine lavage and tissue samples were 59% (26/44) showing great promise in this non-invasive liquid biopsy technique.

Conclusions: Mutation analysis in uterine lavage samples is a promising tool for ovarian cancer prognostics and detection. More research is needed in order to implement uterine lavage use in the clinic.

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76. ANTIMICROBIAL POTENCY OF ESSENTIAL OILS

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The constant increase of multidrug-resistant bacteria poses a threat to human health and underlines the importance of alternative solutions replacing chemical antimicrobials. Plant based materials, including essential oils (EOs), have developed high interest in food industry and healthcare because of their pronounced antimicrobial properties, ecofriendly nature and recognized safe status. EOs can be defined as complexes of hydrophobic and volatile substances extracted from various parts of the aromatic plants such as flowers, seeds, leaves, fruits, roots, etc. The antimicrobial activity of EOs depends on their composition and features of the target microorganisms. The efficacy of EOs against a wide range of microorganisms has been demonstrated; however, the exact mechanism of the action has not been completely elucidated.

The aim of this work was to investigate the antimicrobial effect of EOs on the potentially pathogenic Gram-positive and Gram-negative bacterial species. The antibacterial activity of fourteen EOs was tested using direct contact and evaporation test modes. Differing efficacy of EOs was observed based on their composition. Using direct contact approach, five EOs were found to be effective against all bacteria tested, of which oregano EO had the strongest effect. *Escherichia coli* was inhibited by all EOs studied except that of juniper, and *Pseudomonas aeruginosa* was the most resistant to all EOs tested. The evaporation test showed that volatiles of EOs expressed stronger antimicrobial activity on Gram-positive bacteria than on Gram-negative ones. In the direct contact, a larger proportion of EOs inhibited the growth of bacteria, but their effect was weaker than that

of volatiles. The obtained data will extend the application fields of EOs, increase their potential for solving of food safety problems, preventing multidrug-resistant microbe spreading and maintaining human health.

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77. OVARIAN SENSITIVITY INDEX AS A MEASURE FOR FEMALE INFERTILITY CORRELATES WITH ENDOCRINE DISRUPTIVE CHEMICAL MIXTURE EXPOSURE

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Exposure to endocrine disruptive chemicals (EDCs) such as phthalates, parabens, and per- and polyfluoroalkyl substances (PFASs) potentially interferes with the endocrine system and might affect female fertility. Evidence of such associations, however, remain limited.

In a combined population of 333 women attending fertility clinics in Sweden and Estonia, we studied the associations between EDCs and female fertility, evaluating ovarian sensitivity index (OSI) as an indicator of ovarian response. We measured 59 chemicals in follicular fluid (FF) using isotope dilution LC-MS/MS and detected 11 (5 phthalate metabolites and 6 PFAS) in >90% of women. Associations were evaluated using multivariable-adjusted linear (OSI) models. Bayesian Kernel Machine Regression was additionally applied to evaluate the effect of complex mixtures of correlated environmental chemicals on OSI.

Within a large chemical mixture, we observed significant associations between high levels of several measured EDCs and lower OSI, suggesting their role in interfering with biological processes that lead to infertility and undesirable outcomes in *in vitro* fertilization procedures.

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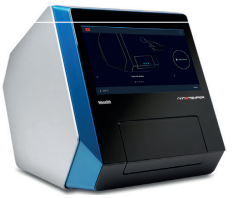

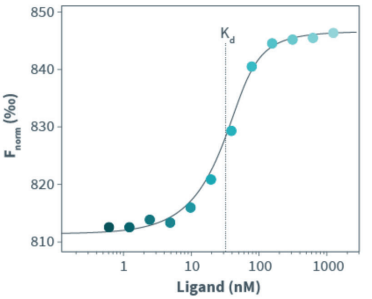

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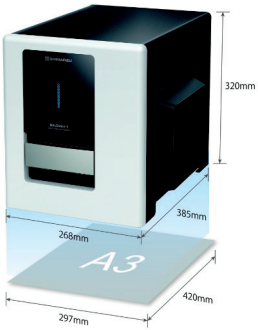

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