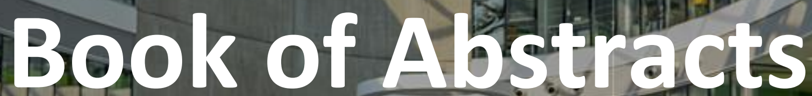

CEITEC PhD Conference

29th May 2023

CEITEC MUNI

CEITEC PhD Conference

29th May 2023
CEITEC MU, Building B11, Auditorium 205

8:30 - 9:00	**Registration**	
9:00 - 9:10	**Welcome**	
B11/205	Pavel Tomančák	
	Kateřina Ornerová	
9:10 - 10:30	**Session 1**	**PRESENTATIONS Life Sciences**
B11/205		CHAIR: Michaela Wimmerová
9:10 - 9:30	Petra Itterheimová *(P. Kubáň)*	Development of portable capillary electrophoresis instrumentation for medical diagnostic
9:30 - 9:50	Zhengyue Zhang *(J. Šponer)*	Collective Variables based enhanced MD promotes the sampling of non-canonical DNA dynamics
9:50 - 10:10	Michaela Medková *(M. Mráz)*	The role of long non-coding RNAs in the microenvironmental interactions of malignant B cells
10:10 - 10:30	Lucie Valentová *(P. Plevka)*	Hunting strategy of phage JBD30 revealed by combination of cryo-electron and fluorescent microscopy
10:30 - 11:00	Coffee break	
B11/205		
11:00 - 12:20	**Session 2**	**PRESENTATIONS Life Sciences**
B11/205		CHAIR: Lukáš Žídek
11:00 - 11:20	Alžběta Dikunová *(R. Štefl)*	Assembly of torpedo termination complexes in thermophilic and mesophilic organisms
11:20 - 11:40	Marek Korsák *(M. Wimmerová)*	Characterization of lectin PluLec from *Photorhabdus luminescens*
11:40 - 12:00	Huma Shakoor *(P. Lukavsky)*	Staufen1 interaction with target 3' UTR and influence of SSM and other domains on Staufen1 oligomeric state
12:00 - 12:20	Jana Faturová *(K. Říha)*	TDM3 is a novel component of Cajal bodies involved in mitotic progression

12:20 - 13:20	Lunch break – participants on their own	
13:20 - 15:00 B11/205	**Session 3**	PRESENTATIONS Life Sciences CHAIR: Jan Havliš
13:20 - 13:40	Evelína Gahurová *(M. Šámalová)*	Exploring the role of cell-type specific expansin overexpression in the control of cell wall biomechanical properties and root growth of *Arabidopsis*
13:40 - 14:00	Katrina Leslie Nicolas *(J. Hejátko)*	Natural genetic variability in multistep phosphorelay as a tool for elucidating drought adaptation in *Arabidopsis thaliana*
14:00 - 14:20	Unnikannan Prabhullachandran *(H. Robert Boisivon)*	Unraveling the process of thermoregulation during the seed development in *Brassica napus* under heat stress
14:20 - 14:40	Sajid Ullah *(J. Hejátko)*	Automated analysis of grain spikes in greenhouse images using deep learning models
14:45 - 15:00 E35/Atrium	Coffee break	
15:00 - 17:00 E35/Atrium	**Session 4**	**POSTER SESSION** CHAIR: Jan Hejátko & Michal Šmída
15:00 - 15:30	**Structural Biology**	
1.	Zuzana Trebichalská *(P. Plevka)*	In-situ cryo-electron tomography of Enterovirus replication
2.	Sepideh Mohammadi Koubjari *(P. Lukavsky)*	3'UTR-mediated regulatory partners in MYC mRNA across different cell lines
3.	Gytis Kučinskas *(J. Hritz)*	Structural analysis of phosphorylated full length Tau protein fibrils without enhancers
4.	Krishnendu Bera *(J. Hritz)*	Conformational changes upon phosphorylation of proline-rich region of tau(210-240) peptide using molecular dynamic simulation
5.	Peter Pajtinka *(R. Vácha)*	Amphipathic helices can sense negative membrane curvature

6.	Sushmita Pal *(R. Vácha)*	Peptide sensitivity on membrane curvature and dependence on lipid unsaturation
7.	Sofia Blasco Puyelo *(R. Vácha)*	Protein motif for bacterial affinity
8.	Mohd Isar *(P. Lukavsky)*	RNA as a therapeutic target
9.	Martin Černý* *(L. Žídek)*	δ-subunit of prokaryotic RNA polymerase C-terminal domain has sequence-specific role in transcription
10.	Subhash Narasimhan *(L. Žídek)*	Integrative structural biology approaches to characterize roles of intrinsically disordered MAP2c and Sigma-A
11.	Filip Melicher *(M. Wimmerová)*	Hexameric lectin from *Photorhabdus laumondii*
12.	Ján Bíňovský *(P. Plevka)*	Baseplate structure and its conformational changes required for genome delivery of S. aureus phage phi812
13	Miroslav Homola *(P. Plevka)*	Structural insight into a virus of climate modulat-ing alga *Emiliania huxleyi*
14.	Veronika Klápšťová *(R. štefl)*	Structural characterization of the interaction between BRCA1-BARD1 and RNA polymerase II
15.	Jitender Kumar *(K. Tripsianes)*	Dual binding mode of Dishevelled PDZ domain
16.	Kateřina Linhartová *(R. Štefl)*	Recognition of RNA Polymerase II C-terminal domain by RPRD2
15:30 – 15:45	**Plants**	
17.	Lucia Baďurová *(J. Hejátko)*	PHD-HD proteins: an enigmatic plant-specific transcription factor family
18.	Alesia Melnikava *(J. Hejátko)*	Cytokinin-induced *dirigent13* gene involves in the lignan production and mediates plant responses to the stresses

19.	Shekoufeh Ebrahimi Naghani *(H. Robert Boisivon)*	Study of the involvement of the PHYB-PIF4 pathway in high-temperature responses during the reproductive phase and embryogenesis of *Arabidopsis thaliana*
20.	Juan Francisco Sánchez López* *(H. Robert Boisivon)*	HSP101 and HSBP mediate gamete and embryo development during thermal adaptation
21.	Surendra Saddala *(K. Říha)*	Unravelling the molecular function of CDM1 zinc-finger protein in meiotic progression in *Arabidopsis thaliana*
22.	Darya Volkava *(K. Říha)*	Deciphering the global proliferative arrest: an elusive link between plant reproduction and longevity
23.	Elena Zemlyanskaya *(K. Růžička)*	mRNA *N6*-adenosine methylation (m^6A) integrates multilevel auxin response and ground tissue development
24.	Yewubnesh Wendimu Seifu *(T. Nodzynski)*	The intracellular auxin homeostasis regulators PIN5 and PIN8 have a divergent membrane topology in *Arabidopsis thaliana* root cells
15:50 - 16:15	**Molecular Medicine**	
25.	Tomáš Reigl *(K. Plevová)*	Exploring patterns in clinical, biological, and molecular data of leukemia patients with CLLue
26.	Ahmadreza Lagzian *(V. Rotrekl)*	DNA damage altered the fate determination in stem cells
27.	Lenka Dostálová *(M. Šmída)*	Genome-wide CRISPR/Cas9 knockout screening revealed genes involved in CD20 regulation in rituximab-resistant cells
28.	Anna Hrčková *(Š. Vaňáčová)*	Searching for new factors involved in RNA tailing and decay
29.	Marie Mądrzyk *(O. Slabý)*	Sequencing of long non-coding RNAs in exosomes in patients with colorectal cancer
30.	Adriana Ladungová *(M. Šmída)*	Investigating the therapeutic potential of clinically-approved drugs for chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML) *in vitro*

31.	Helena Peschelová *(M. Šmída)*	CRISPR/Cas9 technology as a useful tool in the study of chronic lymphocytic leukemia
32.	Pedro Faria Zeni *(M. Mráz)*	The role of long non-coding RNAs in BCR-mediated CLL activation
33.	Karolína Trachtová *(O. Slabý)*	Bioinformatic Pipeline for Comprehensive Analysis of Small RNA-seq Data
34.	Eva Hoferková *(M. Mráz)*	Coculture model with CD40L, IL4 and IL21 for study chronic lymphocytic leukemia proliferation
35.	Veronika Rájecká* *(Š. Vaňáčová)*	An unbiased analysis of interplay between adenosine methylation and editing
36.	Ketty Sinigaglia *(M. A. O'Conell)*	Exploring the interplay between ADAR1, MAVS, and PKR in innate immune responses
37.	Anastasiya Volakhava *(Š. Pospíšilová)*	Uncovering retroelement activity in hematological malignancies
16:15 - 16:20	**Bioinformatics**	
38.	David Čechák *(P. Alexiou)*	Transcriptome-wide prediction of Ago2:miRNA-mRNA interactions using Deep Learning
39.	Kateřina Jurásková *(V. Bystrý)*	Bioinformatics multi-omics approach for data integration from various diagnostic types in pediatric oncology
40.	Kriti Bhagat* *(P. Alexiou)*	Topic not provided by deadline
17:00 - 17:45	**RECEPTION**	
17:45 - 18:15	Announcement of the best presentation/poster	Michaela Wimmerová
E35/Atrium	**Closing remarks**	

*not present in person

PHD-HD proteins: an enigmatic plant-specific transcription factor family

Lucia Baďurová[1], Jan Skalák[1], Jiří Rudolf[1,2], Michal Franek[3], Jan Hejátko[1]

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I am Lucia, a PhD student at the Department of Functional Genomics and Proteomics of Plants at CEITEC, Masaryk University. My current research focuses on exploring the molecular mechanisms governing plant growth and development. Specifically, I am studying the role of the PHD - HD proteins, putative epigenetic readers, whose function in plants is still unknown. I completed my bachelor's degree in Biology and my master's degree in Genetics at Comenius University in Bratislava. During my studies, I conducted research on the molecular mechanism and role of autophagy in virus infection, and on the metabolic reprogramming of host cells during lymphocytic choriomeningitis virus infection. My master's thesis was awarded the rector's prize. I have developed a broad range of technical skills in various laboratory techniques, including cell cultivation and basic virology techniques, plant growth and phenotyping, DNA/RNA analysis, molecular cloning, production of transgenic plants, immunofluorescence assays, confocal microscopy, and protein analysis. Alongside my technical skills, I am adept at time management and excel in teamwork, and I have strong presentation skills. In recognition of my work, I was awarded the Brno PhD Talent 2020. My passion for scientific research is limitless, and I am always eager to tackle new challenges and learn from them. Outside of the lab,

I enjoy taking care of my houseplant collection and savoring a cup of coffee.

Domain modularity is an important feature of transcription factors (TFs) that enabled their functional diversification during evolution. The PHD-HD family is an example of a plant-specific TF family that combines a plant homeodomain (PHD) and homeodomain (HD) architecture. The HD is a DNA-binding domain often found within proteins involved in developmental regulations, while the PHD domain acts as an epigenetic reader recognizing chromatin regions with distinct epigenetic marks. The presence of both HD and PHD within PHD-HD proteins may imply their involvement in the developmental processes via epigenetically mediated transcriptional regulation. Notably, HAT3.1 and PRHA are the only members of the PHD-HD family in *Arabidopsis thaliana*, and their molecular or physiological function is yet to be fully understood.

To investigate the molecular function of HAT3.1, we employed *in silico* prediction tools to search for a nuclear localization signal in its protein sequence and further experimentally confirmed its intranuclear targeting. Interestingly, ectopically expressed HAT3.1-EGFP protein exhibits a speckled nuclear distribution. Moreover, the foci of HAT3.1 protein were shown to colocalize with chromocenters, corresponding to heterochromatic chromosome regions.

To better understand the physiological role of HAT3.1 *in planta*, we analyzed the HAT3.1 expression pattern using a *proHAT3.1::EGFP-GUS* reporter line. Histochemical staining for GUS enzyme activity showed a strong association of its expression with the regions of active cell division, including the meristematic zone of the primary root, emerging lateral roots, and developing true leaves. HAT3.1 expression was also detected during reproductive development, e.g., in the developing flower buds, ovules, and seeds.

As plant development is regulated by both intrinsic and exogenous factors, we analyzed the responsiveness of *HAT3.1* expression to various stimuli. We found that auxin positively regulates *HAT3.1* expression, likely due to the auxin-induced initiation of lateral roots and callus formation. Conversely, salt stress not only reduces *HAT3.1* promoter activity but also negatively affects the primary root length of both *hat3.1* and *prha* mutant lines, as shown by our preliminary phenotyping data.

Taken together, our findings suggest that PHD-HD proteins play a role in early plant developmental processes, and their function can be modulated by exogenous factors, such as salt stress. Further studies on this unique TF family could provide insight into novel regulators of plant growth and development.

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Conformational changes upon phosphorylation of proline-rich region of tau(210-240) peptide using molecular dynamic simulation

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I am Krishnendu Bera pursuing my PhD under the supervision of doc. RNDr. Mgr. Jozef Hritz. I am working on multiple protein-peptides and protein-small organic molecule interactions associated with neurodegenerative diseases. All the proteins I have used in my studies have a role in neurodegenerative diseases. In my first project, I am exploring computationally how hyperphosphorylation modulates the biologically relevant interactions of proline-rich region of various lengths of tau and rd-hTH1 interact with 14-3-3ζ protein. In my second project, I am computationally screening FDA-approved drug molecules to modulate the dimer monomer equilibria of 14-3-3ζ protein and the effect of two food colour agents forming lysozyme amyloid fibril. In my last project, I tried to see the refolding and unfolding rate of human CRABP I protein modulated by PEG, the crowding agent. CRABP I protein is responsible various neurodegenerative diseases such as amyotrophic lateral sclerosis, spinal muscular atrophy and late-stage age-related macular degeneration. These computational predictions are being validated experimentally by my colleagues in my lab or by different collaborators across the globe.

The conformational dynamics of intrinsically disordered proteins (IDPs) regulated by post-translational modifications such as phosphorylation is challenging to elucidate. A well-known IDP Tau is found hyper-phosphorylated in Alzheimer's disease (AD) in humans [1]. The proline-rich motif of Tau(210-240) peptide directly interacts with proteins such as BIN1, 14-3-3 etc. BIN1 is a major genetic risk factor for AD. Phosphorylation of T217

within the Tau(210–240) peptide led to a 6-fold reduction in the affinity, while single phosphorylation at either T212, T231, or S235 had no effect on the interaction. Nonetheless, combined phosphorylation of T231 and S235 led to a 3-fold reduction in the affinity, although these phosphorylations are not within the BIN1 SH3-bound region of the Tau peptide. But the four phosphorylation reduced the binding affinity by 12-fold. The microsecond time scale, all atoms molecular dynamic (MD) simulation studies have been performed for apo and phosphorylated (pT212, pT217, pT231 and pS235) Tau peptide(210-240) using three different temperature variants (278K, 298K and 310K) and two different force field parameters (AMBER99SB-ILDN and CHARMM36m) with TIP4PD water model as these force field parameters combine with water model worked the best for IDPs from our group previous study [2]. These four-phosphorylations cause an increase in compactness. The strong salt bridges may alter the binding of associated proteins like 14-3-3 with Tau, forming nearby lysine and arginine due to phosphorylation. Phosphorylation induces a strong structural transition, with Tau(210–240) favouring a bent conformation. The MD simulation results were verified using NMR experimental parameters like chemical shift, 3J-coupling [3].

Other PhD projects

Other than this project, I am also working on multiple protein-peptides and protein-organic molecule interactions associated with neurodegenerative diseases. All the proteins I have used in my studies have a role in neurodegenerative diseases. I am exploring computationally how hyperphosphorylation modulates the biologically relevant interactions of proline-rich region of various lengths Tau and rd-hTH1 interact with 14-3-3ζ protein, and two manuscripts will be summarized shortly. I have studied the impact of small organic molecules in lysozyme amyloid fibrilization [4, 5]. During the COVID-19 pandemic, I have worked on inhibitor designing of two proteases of COVID-19 [6, 7], which helped me to design the pipeline for screening FDA-approved drug molecules to modulate the dimer monomer equilibria of 14-3-3ζ protein and the experimental part is now carried out in our lab. I also am trying to see the refolding and unfolding rate in the presence of PEG1000 and PEG2000 on human CRABP I protein, which is one of the responsible proteins for Amyotrophic Lateral Sclerosis [8, 9]. These computational predictions are being validated experimentally by my colleagues in my lab or by different collaborators.

Computational resources were supplied by the metacentrum and IT4 Innovations National Supercomputing Center (OPEN-17-7), project (e-INFRA CZ 90140 and LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructures. KB and JH were funded by the Ministry of Education, Youth, and Sport of the Czech Republic (MEYS CR), grant number LTAUSA18168 (Inter-Excellence Inter-Action), Czech Science Foundation [GF20-05789L] and European Union Excellence Hub (101087124). KB is also supported by Brno Ph.D. Talent Scholarship – funded by the Brno City Municipality, Brno, Czech Republic. I am acknowledging Prof. Isabelle Landrieu and her group for the experimental part of tau210-240.

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Baseplate structure and its conformational changes required for genome delivery of S. aureus phage phi812

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Jan Binovsky is a PhD student in Structural Biology at the Central European Institute of Technology. His research in the group of Prof. Plevka focuses on the structural biology of viruses, emphasizing the mechanisms of viral entry and replication. Jan received his M.Sc. in Biochemistry from Masaryk University, where he conducted his undergraduate research with Prof. Wimmerova, studying lectins of pathogenic bacteria. During his undergraduate studies, Jan spent six months at the National Center for Scientific Research (Grenoble, France) as a research intern, gaining experience in protein biochemistry and X-ray crystallography. As a graduate student, he visited Novo Nordisk Center for Protein Research (Copenhagen, Denmark) for a short internship, where he contributed to a cryo-electron microscopy analysis of viruses while acquiring new insights into the biochemistry of transmembrane proteins. In addition to his research, Jan participated in various outreach events to promote science and engage with the community.

Staphylococcus aureus, a human pathogen, is a major contributor to the global antimicrobial resistance problem. Phage phi812, which can infect up to 90% of S. aureus isolates, is a promising phage therapy agent. We aimed

to investigate the molecular mechanisms of phage phi812 infection to maximize the potential of this therapeutic approach.

To elucidate the initial stage of phage phi812 infection, we conducted a structural study of the phi812 baseplate. The phage baseplate was reconstructed in both pre- and post-contraction states using a combination of cryo-electron microscopy and X-ray crystallography. Our analysis revealed molecular composition of the baseplate core and six rigid baseplate arms with three types of receptor binding proteins. Upon host cell engagement, the conformational changes of the receptor-binding proteins propagate through the baseplate core to the tail, resulting in the contraction of the tail sheath. The process of tail sheath contraction drives the tail tube through the cell wall, which is degraded by phage enzymes found at the tip of the tail tube.

By comparing the two distinct baseplate states, we have described the initial stage of phage phi812 infection at the molecular level. Our study presents the first detailed structure of a contractile phage baseplate infecting a Gram-positive bacterium. Thus, our findings provide a framework for engineering phage particles to combat S. aureus infections in humans.

We acknowledge the Cryo-electron microscopy and tomography core facility and the Biomolecular interaction and crystallization core facility of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127).

Protein motif for bacterial affinity

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I am a PhD student in the field of computational biophysics. My research activities involve mainly running and analyzing molecular dynamics simulations. Currently I am working on the development of antimicrobial peptides as well as on designing nanoparticles able to induce fusion.

One of the top 10 public health concerns to humanity, according to the World Health Organization, is antibiotic resistance. It is therefore necessary to develop new ways to treat bacterial infections. Antimicrobial peptides (AMP) are a promising option to tackle this problem. Due to their mechanism of action, which usually disrupts the cell membrane of bacteria, their use is less likely to lead to the development of resistant bacteria. Nevertheless, most AMPs not only are active against bacteria but also toxic to mammalian cells. Therefore, we need to develop AMP sequences with higher selectivity for bacteria and reduced toxicity for human cells.

In this work, we develop peptide sequences that would selectively adsorb to bacterial membranes. Our approach is based on free energy calculations of amino acid analogs along two membrane models which mimic the cell membrane of bacteria and the cell membrane of mammalian cells. We used all-atom molecular dynamics simulations to calculate the potentials of mean force of each amino acid analog, as well as of some relevant interactions between analogs such as salt-bridges or interactions between aromatic groups. We then used a genetic algorithm to predict sequences whose free energy of adsorption would be lower for bacterial membranes than for mammalian membranes.

Transcriptome-wide prediction of Ago2:miRNA-mRNA interactions using Deep Learning

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David Čechák specializes in the application of Machine Learning in Biology, his research focuses on the classification of genomic sequences using Deep Learning. He has delivered several workshops focused on teaching Machine Learning at the European Conference on Computational Biology (ECCB). His experience ranges from computer vision to web development. His approach to computational biology is backed by a foundation in Computer Science and Artificial Intelligence.

Understanding the interactions between Argonaute2 (Ago2), microRNAs (miRNAs), and messenger RNAs (mRNAs) is critical to unravelling the complex mechanisms underlying gene regulation. This research proposes a comprehensive pipeline capable of scanning the transcriptome to predict whether specific miRNAs target an mRNA and narrow down their binding locations.

The modular pipelineand encapsulates a data preparation component, a neural network (NN) model, methods to scan transcriptoms and produce fold change score based on a given miRNA, and a module comparing our solution results with existing methods.

The NN model aimes at predicting the binding of a pair of Ago2:miRNA to mRNA, thus facilitating an accurate analysis of gene expression regulation. The development of the model involves the creation of suitable datasets using Ago2:miRNA binding site data from various sources, the training of the NN model to predict binding affinities. Binding site scores will be refined based on additional characteristics such as evolutionary conservation. We narrow down the binding sites using deep learning explainablity methods such as counting the SHAP (SHapley Additive exPlanations) values.

Our project compares simple methods and deep learning approachs to scan transcriptomes and predict the fold change score of an mRNA in response to a given miRNA. The model takes into account the sequence complementarity between the miRNA and mRNA. It generates a probability score reflecting the likelihood of a given miRNA binding the mRNA, which can be correlated to or converted into a fold change score.

This research has been supported by Grantová Agentura České republiky, 19-10976Y.

δ-subunit of prokaryotic RNA polymerase C-terminal domain has sequence-specific role in transcription

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Dear reader, my name is Martin Černý and I am a PhD student in the Protein Structure and Dynamics group of prof. Mgr. Lukáš Žídek, PhD who is also my supervisor. There I focus on the structural elucidation and interplay between transcription factors and DNA dependent RNA polymerase and their complexes with a focus on these processes in Bacillus subtilis and Mycolicibacterium smegmatis. Before delving into the field of structural biochemistry, I obtained a bachelor's and master's degrees in Experimental Biology at the Faculty of Science, Masaryk University. Previously, I specialized in Microbiology and Biotechnology, part of which I studied the ecology of methanogenic archaea. During this time, I was a member of the Laboratory of Anaerobic Microorganisms under the supervision of Assoc. Prof. MonikaVítězová, PhD, researching the biotechnological potential of methanogenic archaea to produce biogas for energetic purposes in aquifer environments. However, my personal focus was on the human gut archaeome with a focus on idiopathic bowel diseases. During this, I became well-versed in microbiological methodology, strict anaerobic cultivation techniques, microscopy, and qPCR. My actual research interests are in the domain of transcription factors in gram-positive bacteria with the employment of NMR spectroscopy and cryo-electron microscopy as the main means of structural characterization approach.

Unfortunately, I am not present due to a research stay. If you are interested in my or my group work, feel free to email me, or reach out to my colleagues.

Keywords: transcription initiation, RNA polymerase, *Firmicutes*, intrinsically disordered proteins, NMR, Cryo-EM

In *Bacillus subtilis*, the additional subunit of the RNA polymerase (RNAP) - δ is responsible for transcription regulation [1]. δ destabilizes open-promoter complexes and it affects the binding of σ-factors to RNAP [2]. It is comprised of 2 domains, of which the C-terminal one (δCTD) is negatively charged and intrinsically disordered. It contains K-D/E motif comprised of 7 lysines, preceding the negatively charged tail, which is crucial for RNAP activity. However, in Staphylococci, the K-D/E tract is missing [2]. Our goal was to elucidate what is the relation between δCTD sequence and its function as well as how δ interacts with RNA polymerase.

Here we present the first steps in answering these questions. First, we characterized the bio-physical properties of *S. aureus* δ and compared it with δ of *B. subtilis*. Second, NMR experiments were measured. And last, the late-stationary phase RNA polymerase complex was investigated and a 4.11Å resolution cryo-EM map was obtained. At present time, the σ^A+δ+RNAP reassociation studies are ongoing and the NMR data analysis of staphylococcal δ is on the way.

The research was supported by Czech Science Foundation Grant No. 22-12023S. We hereby thank Josef Dadok National NMR Centre of CIISB, Cryo-electron microscopy and tomography CF CEITEC MU of CIISB, Biomolecular Interactions and Crystallography CF of CIISB for measurements and provided assistance

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Assembly of torpedo termination complexes in thermophilic and mesophilic organisms

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Alžbeta Dikunová studied Experimental biology (Bc.) and Molecular biology (Mgr.) at Masaryk University. Currently, she is in her 4th year of PhD at Ceitec PhD school. As a member of Richard Štefl research group, Alžběta is focused on deciphering the structures and proteins interactions of RNA processing machineries, especially in the area of termination of RNA polymerase II transcription.

Proper organization of transcription is essential for expression and regulation of genes in all organisms. In the past two decades has been proved that not only initiation, but also properly timed and highly regulated termination, play important role in controlling the fate of mRNA. In eukaryotes, two processes are required for termination of protein-coding genes: the production of defined RNA and the disengagement of RNA polymerase II (RNAPII) from DNA template. Both processes are connected, and their detailed molecular mechanisms are still poorly understood. In mesophilic yeast, *Saccharomyces cerevisiae*, there are at least two model pathways how termination of mRNA coding genes is organized: allosteric (antiterminator) and torpedo models. The allosteric model involves conformational changes in the elongation complex and binding of termination factors followed by dissociation of RNAPII. The torpedo model proposes that the unprotected free 5'-end of the mRNA transcript is digested by nuclease until it collides with RNAPII, leading to dissociation from the template.

Indeed, recent studies in yeasts showed that exposed and unprotected free 5'-end of RNA serves as an entry point for Rat1, 5'-3' exonuclease, stimulated by its cofactor Rai1, forming Rai1/Rat1 (RR) complex. However, the exact mechanism of how the RR complex is recruited

and how RNAPII is then released from the remaining RNA transcript during termination is unknown. Findings also suggest that the torpedo termination complex is recruited by Rtt103, recognizing Ser2 and or Thr4 phosphorylation marks of RNAPII which are high at the polyA site. This allows us to hypothesize that Rtt103 recruits 5’-3’ RNA termination machinery in order to trigger RNAPII release from the DNA template.

Due to the variations in Rai1/Rat1/Rtt103 complexes in mesophilic (yeast) and thermophilic organisms, we set out to investigate whether and how these complexes assemble in *Saccharomyces cerevisiae* and *Chaetomium thermophilum*. Using a combination of structure biology techniques including cryoEM, nuclear magnetic resonance spectroscopy (NMR), small-angle X-ray scattering (SAXS) and cross-linking mass spectrometry (XLMS) we reveal differences of how the two torpedo complexes assemble in yeasts and thermophiles.

In thermophilic organisms, the protein Rtt103 interacts with the RR exonuclease complex using highly structured elements. In contrast, yeast relies on the unstructured regions of Rtt103 for its interaction with the RR complex. This observation implies that thermophilic organisms have evolved protein-protein interfaces that favor the presence of highly structured elements, whereas mesophilic organisms prefer the utilization of unstructured elements.

Genome-wide CRISPR/Cas9 knockout screening revealed genes involved in CD20 regulation in rituximab-resistant cells

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Lenka Dostálová is a 3rd year PhD student in the Molecular Medicine program at the Faculty of Medicine. She has been working in the Functional Genomics group under the supervision of Dr Michal Šmída, focusing her research on the regulation of CD20 antigen in B-cell malignancies. CD20 is used as a target of monoclonal antibodies in the therapy of B-cell malignancies; however, malignant cells tend to decrease its levels on the cell surface. Lenka aims to uncover mechanisms that could potentially reverse CD20 downregulation and thus enhance the efficacy of anti-CD20 monoclonal antibodies.

CD20 antigen has been used as a target of monoclonal antibodies (mAb) such as rituximab (RTX) in the therapy of B-cell malignancies for more than two decades. However, malignant B cells downregulate CD20 on their surface, resulting in mAb resistance and therapy failure. Therefore, it is crucial to investigate the CD20 regulation to enhance the efficacy of anti-CD20 mAb. This project aimed to perform CRISPR/Cas9 knockout screening to identify genes whose disruption restores CD20 surface expression.

To create a model mimicking the situation in patients who have developed resistance to mAb therapy, we generated RTX-resistant CD20-low B-cell line by chronic exposure to rituximab. These cells were transduced by the GeCKO lentiviral library to obtain a collection of single-gene knockouts.

After 2.5-week cultivation, the top 5% of cells with the highest expression of CD20 were sorted out. Using next-generation sequencing, we identified gene knockouts responsible for CD20 upregulation.

CRISPR/Cas9 screening revealed several genes whose disruption increased CD20 expression. CSK, encoding a negative regulator of Src kinases, as well as *PTEN*, a well-known tumor suppressor, were among the top hits. These two genes are involved in the B-cell receptor (BCR) pathway – an essential pathway in B cells. Interestingly, we identified four genes *SSR1-4* encoding all four subunits of the TRAP complex, an endoplasmic reticular complex involved in protein translocation across ER membrane. *STT3A*, encoding the catalytic subunit of oligosaccharyltransferase, was another ER-associated gene revealed by the screening. These results indicate that both BCR signalling and ER play an important role in CD20 regulation. Selected genes were validated and the mechanism of their function is being investigated. The understanding of underlying mechanisms could provide a way for a potential enhancement of anti-CD20 mAb therapy.

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Study of the involvement of the PHYB-PIF4 pathway in high-temperature responses during the reproductive phase and embryogenesis of *Arabidopsis thaliana*

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This is Shekoufeh Ebrahimi. I am a 5th year PhD student from Helene Robert Boisivon lab. My PhD project goal is to discover high the genetic pathway responsible for temperature response during inflorescence development in Arabidopsis thaliana.

Global climate change is causing an increase in temperatures, which significantly affects various developmental processes in plants, such as growth and flowering. Using *Arabidopsis thaliana* as a model organism we have gained substantial knowledge of signaling and response mechanisms during plant exposure to elevated temperatures. A substantial amount of data has been gathered to elucidate the role of hormones and molecular chaperones, such as Heat shock proteins (HSPs), in this temperature response in vegetative tissues. In this study, we analyze the function of the PHYB-PIF4 pathway, known for controlling high-temperature stress in the seedling during reproductive development and seed production. Some mutants [*phyb*, *pif4*, and quadruple *pif1/3/4/5* mutants (*pifq*)] and a *PIF4* over-expression line have been studied. Embryo phenotyping reveals that heat stress causes suspensor shortening. Further analysis is required to elucidate if PIF4 is involved in this response. Besides dwarf suspensor, we observed some defects in the embryo proper division at high temperatures, such as extra division in the hypophysis. The severity of the heat stress effect was roughly the same in all the studied lines. Despite RNA-seq data and

histochemical GUS assays suggesting that *PIF4* is not expressed during embryonic developmental stages, we show that elevated temperature increases *PIF4* expression in the seed integuments. Anther-phenotyping indicate that pif4 and pifq mutant lines are tolerant to temperature and do not exhibit any anther abnormalities. In contrast, wild-type plants, phyb, and 35S:PIF4 lines grown at higher temperatures showed reduced anther size and male fertility. Ovule phenotyping indicates that over-expression of PIF4 at normal conditions mimics the effect of heat stress on the wild type. Moreover, exposure of phyb mutants and p35S:PIF4 lines to heat stress results in defects in approximately 90% of produced ovules, while this percentage decreases to around 40 in wild-type, pif4, and pifq mutant lines. GUS assays indicate that elevated temperatures also boost PIF4 expression in the ovule and anther. Microscopy analysis of YUCCAs reporter lines showed that temperature also alters the expression level of some auxin biosynthesis genes (*YUCCA4*, *YUCCA8* and *YUCCA10*) in the seed coat and ovule. We hypothesize that higher *PIF4* expression in these tissues affects *YUCCCA* expression, leading to abnormal development. We will perform further experiments like RNA-seq to find PIF4 target genes at high temperatures in ovules.

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TDM3 is a novel component of Cajal bodies involved in mitotic progression

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Meet Jana Faturová, a highly motivated and hardworking PhD candidate in Life Sciences at CEITEC MU in KR Group. Her research area revolves around studying plant cell division and stress response mechanisms, focusing on molecular biology, genetics, and cellular biology. Thanks to previous studies at Charles University in phycology and botany, Jana has developed a unique ability to connect different biological fields. She is passionate about exploring complex biological systems and dedicates her free time to admiring the natural world. Jana firmly believes that spending time in nature helps her stay grounded and find the inspiration necessary for her work. If you are interested in any questions about her work or some tips for hiking, you can contact her at jana.faturova@ceitec.muni.cz.

Bimolecular condensates, such as cytoplasmic stress granules, P-bodies, or nuclear Cajal bodies, are important in RNA biogenesis and gene expression. In our previous work, we discovered that TDM1, a plant-specific protein expressed exclusively during meiosis, associates with P-bodies and contributes to the termination of the meiotic program by mediating inhibition of translation (Cairo et al. 2022). TDM1 belongs to a protein family with five homologous in Arabidopsis thaliana, two of which are implicated in transcriptional regulation of glucosinolates biosynthesis, while the function of the other two is unknown. Here we describe TDM3, another member of this family. We found that TDM3 is expressed in dividing somatic cells from the S-phase till telophase and appears to be rapidly degraded after M-phase.

Notably, in contrast to TDM1, TDM3 has nuclear and nucleolar localization, which is associated with Cajal bodies, nuclear condensates, primarily involved in the biogenesis of small nucleolar RNAs and splicing. Arabidopsis mutants deficient in TDM3 are smaller and, at some point, defective in mitosis compared to WT. Together, our data suggest that proteins of the TDM family play an essential role in biomolecular condensates involved in RNA biogenesis and regulation.

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Exploring the role of cell-type specific expansin overexpression in the control of cell wall biomechanical properties and root growth of *Arabidopsis*

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Evelína Gahurová is a PhD student at CEITEC PhD school at Masaryk University. Currently, she works at the Department of Experimental Biology and her supervisor is Dr. Šámalová. Her PhD topic is the role of expansins and plant hormones in the control of cell wall properties and root apical meristem development in Arabidopsis.

The cell wall (CW) plays a crucial role in plants since it defines cell shape, enables cell connections, and provides a protective barrier against pathogens and environmental factors. The main constituents of the primary cell wall are cellulose and hemicellulose polysaccharides, which provide the basic mechanical strength, and pectins that can modify the viscoelastic properties of the matrix. Cellulose and lignin are the most abundant polymers on Earth and their modifications have an application in a wide range of industries such as food, paper, textiles, fibers, and pharmaceutical industries. Lignocellulosic biomass can be potentially used as second-generation biofuels and as an alternative to fossil fuels.

Expansins (EXPAs) are known to disrupt non-covalent bonds in the cell wall structure without structural changes of the CW and EXPAs do not possess a hydrolytic activity. EXPAs are activated during CW acidification triggered by a number of stimuli through the plasma membrane H+-ATPase

proton pumps. EXPAs are involved in the CW loosening by mediating the slippage of carbohydrate polymers at load-bearing elements of the CW. Nevertheless, their molecular mode of action enabling cell wall expansion remains unclear.

Our previous results suggested that spatial-specific distribution of expansins and disruption of fine-tuned pH and strain-stress optimum leads to growth arrest of *Arabidopsis* roots. Therefore, we propose that tightly controlled spatiotemporal specificity of expansin expression and hormonal-mediated pH distribution within the root apoplast plays an important regulatory role in controlling root growth and development in *Arabidopsis*.

We induced ectopic expression of α-expansin 1 (EXPA1), in each individual layer of the root apical meristem (RAM) using cell type-specific activators of the chemically inducible transcription activation system pOp6/LhGR. We measured the size of the root and RAM but interestingly, we did not find any significant differences.

Furthermore, we plan to follow closely the growth of selected lines after applying hormonal treatment and pH changes using confocal laser scanning microscopy. Moreover, we will try to lower the pH in specific cell types by overexpressing the H+-ATPase using the inducible system above and investigate the dynamics of root growth and finally associated changes in the cell wall biomechanical properties.

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Coculture model with CD40L, IL4 and IL21 for study chronic lymphocytic leukemia proliferation

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Eva Hoferková is a PhD student in a research group of Dr. Marek Mráz at CEITEC Masaryk University in Brno. Eva specializes in the development of a patient-derived mouse model for chronic lymphocytic leukemia, based on a novel coculture system. She studied molecular biology and genetics at Masaryk University and stayed in Brno also for her PhD. When she is not in the laboratory, Eva spends her time playing sports, growing home plants, and baking sweets for her sweet-toothed colleagues.

Chronic lymphocytic leukemia (CLL) cells exhibit dynamic trafficking between the quiescent and activated states in the lymphoid microenvironment, where they proliferate in response to signals from CD4+ T cells. These signals can be mimicked *in vitro* with artificial stimulation by CD40L, IL4, and IL21. In this study, we generated a panel of HS5-derived cell lines expressing T cell factors, which induce CLL proliferation and support CLL survival.

Using a coculture model, microenvironment-targeting drugs with potential effects on CLL cell proliferation were tested. Specifically, clinically used inhibitors including ibrutinib and idelalisib, and some of them were found to significantly inhibit CLL proliferation, with a reduction of up to 50% in the number of proliferative cells observed. The effect was not influenced by increased apoptosis.

To extend the potential application of the prepared coculture model, we downscaled the culture into a specific microwells seeded with a number of cells that decreased 800× compared to a standard culture in a 12-well plate. Under such conditions, CLL cells in microwells were induced to proliferation similarly to those in the standard culture plate.

On the other hand, we attempted to upscale the coculture into a mouse model. Transplantation of purified CLL cells into the NSG mice conditioned with HS5 cells led to a lymphomagenesis in mice. The origin of the tumors was verified by sequencing and comparing Ig rearrangements in tumor cells and the original CLL samples. Different rearrangements were detected in most cases. The tumor B-cells showed indications of EBV-driven growth, which was confirmed by detecting the EBNA1 gene in DNA lysates from mouse spleens.

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Structural insight into a virus of climate modulating alga *Emiliania huxleyi*

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I am a PhD student in the Structural virology group interested in environmentally important alga viruses.

Emiliania huxleyi is the most abundant marine alga whose cells are covered by calcite disks. By reflecting light, the calcite disks reduce the absorption of sunlight in ocean water, which impacts planetary climate[1]. Large nucleocytoplasmic DNA viruses, including *E. huxleyi* virus 201 (EhV-201), are a major factor limiting the population density of *E. huxleyi*[2]. Despite the impact of EhVs on the Earth's climate, there is limited information about their structure and replication.

Here we show that EhV-201 virions are covered by the inner membrane, capsid, and outer membrane. Upon infection EhV capsids remain attached to cell surface whereas the genomes are delivered into the cytoplasm. Replication of EhV-201 results in the formation of cytoplasmic virus factories, which are the sites of virion formation. Capsid assembly is initiated at the surface of endoplasmic reticulum-derived membrane segments. The assembling capsid bends and engulfs the membrane, resulting in the formation of a membrane sack. The virus genome is packaged through an opening in incompletely assembled cap-sid. EhV-201 capsids are uniform in

size and assemble according to the rules of quasi-icosahedral symmetry; however, contacts between the capsid proteins are flexible, and most of the particles become deformed. Genome-filled particles acquire the outer membrane by budding into intracellular vesicles. Most EhV-infected cells lose their polysaccharide envelopes, which enables the release of virions by exocytosis or lysis of the infected alga. Structural characterization of EhV infection, a process that impacts the Earth's climate, may inspire means to influence it.

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Searching for new factors involved in RNA tailing and decay

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Anna Hrčková is a 3rd year PhD student in the Biomedical Sciences programme of the Medical faculty and CEITEC Masaryk University. She works in the group of RNA quality control under the supervision of professor Štěpánka Vaňáčová. She is looking for new protein players in RNA degradation pathways in human cells as well as uncovering new mechanisms of RNA metabolism in eukaryotic single cell flagellate Euglena. She has experience with recombinant protein purifications, biochemical assays, and human cell lines handling. She is a holder of Brno PhD talent grant 2020 supported by Brno municipality.

Gene expression in eukaryotes is regulated by diverse mechanisms. The key regulators include the production and stability of coding and noncoding RNAs. Every primary RNA transcript must undergo further processing and modifications, often involving complex machineries. The second main parameter for RNA metabolism is its stability, which defines the time frame within which it performs its role.

Nontemplated RNA tailing plays critical roles in RNA processing, specificity as well as stability. Dysregulation of RNA tailing leads to disease. Here we focus on nontemplated addition of one or more uridines (oligo(U)) which is mediated by the activity of terminal uridyltransferases (TUTAses). The target specificity and function of oligoU is established by TUT associated cofactors. DIS3L2 is a 3’ to 5’ exoribonuclease recognising RNA molecules uridylated by TUT enzymes and degrading them, establishing the TUT-DIS3L2 surveillance (TDS) pathway. [1][2] However only scarce number of other factors employed in TDS is known to this date.

We use proximity labelling miniTurboID [3] to identify additional cofactors in this pathway. We use HEK293T cell line with inducible expression of proteins of interest fused with promiscuous biotin ligase to biotinylate proximal proteins. The biotinylated proteins are purified with streptavidin and identified by quantitative mass spectrometry.

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RNA as a therapeutic target

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My primary focus is on designing methods to exploit RNA as a potential therapeutic target. I am currently in my third year of doctoral studies. With a background in biochemistry and biophysics, I am targeting 3'UTR mRNA of several undruggable oncogenes and non-oncogene addiction genes with drug-like small molecules in a high-throughput setup. The idea is to disrupt RNA-protein interactions with small molecules, thereby downregulating the gene expression of disease-related proteins in systemic and cancer-related pathologies. My goal is to deliver novel and translatable results for future medication.

The majority of currently available drugs target proteins, but a large proportion of proteins, around 80%, are considered undruggable; therefore, selecting another suitable candidate is crucial for treating many systemic and cancer-related pathologies. Around 75 % of the human genome is transcribed into RNA, while only a small fraction (~3%) of it translates to protein. Hence, RNA can be exploited as a potential therapeutic target. Specifically, the 3' untranslated regions (3'UTRs) of RNAs which play a crucial role in mRNA stability and gene expression regulation, have distinct secondary and tertiary structures that interact with proteins within cells. In this study, the goal is to identify small drug-like molecules that can target the 3'UTRs of several non-druggable oncogenes and non-oncogene addiction genes, such as MYC, KRAS, HSF1, CDK12, NRF2, etc.

A library of specialized heterocyclic compounds representing drug-like small molecules was screened against 120 nucleotide fragments of the MYC mRNA 3'UTR using a high-throughput fluorescence-based anisotropy assay (FA). Several fragments showed significant changes in fluorescence anisotropy when mixed with small molecules, indicating potential binding and conformational changes in the RNA structures. Selected RNA fragments were subsequently screened using Surface Plasmon Resonance (SPR), a highly sensitive biophysical technique where RNA is immobilized, and small

molecules are flowed across the surface. Significant binders were identified, indicating the potential of these small molecules to interact with the RNA targets.

Our screening approach leverages the conformational changes in RNA fragments upon small molecule binding. Further characterization of these interactions will enable us to create a library of RNA motif-small molecule interaction pairs that can be utilized to target other mRNAs. These interaction pairs will aid in identifying lead compounds capable of modulating mRNAs and mRNA-protein complexes within cells. Disrupting RNA-protein interactions with small molecules can result in the downregulation of gene expression of disease-related proteins in systemic and cancer-related pathologies, which eventually has potential therapeutic implications.

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Development of portable capillary electrophoresis instrumentation for medical diagnostic

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Petra Itterheimová is a 4th year PhD student of Life Sciences doctoral degree programme at CEITEC PhD School. She received a bachelor's degree in chemistry from Masaryk University and master's degree in analytical chemistry at the same university. Her current field placement is with the Czech Academy of Sciences at the Department of Bioanalytical instrumentation. She focuses on low-cost instrument development for capillary electrophoresis analytical technique and its applications for medical diagnostic purposes.

Capillary electrophoresis (CE) is a widely used analytical separation technique. Several commercial instruments are available, however their cost and dimensions might be limiting for many specific applications. Inexpensive in-house built instruments that are based on the open-source hardware, following the general acceptance of open-source paradigm in science, are being developed more and more often. In our work, we focused on developing two simple and cheap components of an in-house built CE instrument. At first, we have constructed small and simple data acquisition (DAQ) devices based on Arduino Nano microcontroller and various analog-to-digital converter

modules with resolution in the range from 16 to 24 bit. For the CE system with contactless conductivity detection, the best results were obtained with the developed 24-bit DAQ device, the performance of which was comparable to a commercial, high-end 24-bit converter (ORCA 2800). In the second project, an autosampler for a modular CE system was constructed. The entire system is driven by an Arduino Mega microcontroller, which manages hydrodynamic sample injection, as well as its control enabled by a keypad and LCD display. The functionality of the autosampler was tested and the results were comparable to analyses, where hydrodynamic injection was carried out manually. Application for such an in-house built CE instrument may be for instance the ion analysis of sweat samples to diagnose cystic fibrosis, which is based on elevated ion ratio ((Cl-/K+) or ((Cl- + Na+)/K+)) values for patients in comparison to healthy people. In order to obtain a suitable sweat sample, a novel, simple and 3D-printed sampling device was also developed and tested in clinical practice.

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Bioinformatics multi-omics approach for data integration from various diagnostic types in pediatric onkology

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My name is Kateřina Jurásková and I am a third-year doctoral candidate in the Life Sciences PhD Program. I work as a bioinformatician in the Bioinformatics Core facility at CEITEC, where I specialize in bio-omics approaches, focusing on genomics and transcriptomics. My primary goal is to develop innovative bioinformatics methods for data integration from various diagnostic types in the field of clinical oncology, which will help improve our understanding of complex interactions and patterns governing biological systems.

Next Generation Sequencing (NGS) techniques offer a comprehensive approach to analyzing the tumor molecular structure and the human genome. When combined with a multi-omics approach, NGS provides valuable insights on multiple levels. The challenge lies in integrating these diverse NGS data sources into a unified model that enhances the prediction and confirmation of mutagenic variants.

In the field of pediatric oncology, we have developed a bioinformatics pipeline that utilizes a multi-omic approach, incorporating variant calling, fusion gene detection, expression profiles, and DNA methylation profiles. Each of these analyses provides valuable insights into the structural changes occurring in human tissues, contributing to a comprehensive understanding of pediatric solid tumors. By integrating these diverse data sources, our bioinformatics tool aims to enhance variant prediction and confirmation,

simplify therapeutic planning, and increase confidence in patient stratification for existing diagnostics. This pipeline caters to the specific needs of our genetic clinicians and has the potential to significantly contribute to the personalized treatment of children with solid tumors.

To ensure reproducibility, easy control, scheduling, and self-sufficiency in requirements installation, the implementation of our pipeline relies on the Snakemake workflow manager. Additionally, the results will be interactively visualized using JavaScript libraries such as React and D3, providing intuitive and insightful representations of the analyzed data.

We would like to express our gratitude to the Core Facility Bioinformatics of CEITEC Masaryk University for their contribution in providing the scientific data presented here.

Structural characterization of the interaction between BRCA1-BARD1 and RNA polymerase II

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Veronika Klápšťová is a PhD student in the research group of Richard Štefl. Her research project is focused on investigating the cross-talk between transcription and DNA repair, two key cellular processes, using biochemical and structural approaches.

Incomplete transcripts resulting from the transcription of a damaged DNA may be toxic for the cells, and, therefore, transcription under DNA damage conditions ought to be tightly regulated. Although the coordination of transcription and DNA repair is crucial for the cell viability, the underlying mechanisms are still not fully understood. One of the possible players involved in regulation of transcription under DNA damage conditions is the BRCA1-BARD1 complex, as it interacts with phosphorylated C-terminal domain of RNA polymerase II (RNAPII), and it reads the epigenetics marks present on the chromatin after DNA damage (K15 ubiquitination on the histone H2A). The structural characterization of the complex between RNAPII, BRCA1-BARD1 and the ubiquitinated nucleosome core particle, as well as the description of the conditions under which it is formed will help us to analyze its function in the regulation of transcription after DNA damage. This, in turn, will help us to understand how cells coordinate transcription and other competing processes on DNA, such as DNA repair or replication.

Characterization of lectin PluLec from *Photorhabdus luminescens*

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Marek Korsák is a PhD candidate in the Glycobiochemistry Group. His scientific focus is on the structure-functional characterization of proteins involved in host-pathogen interactions, mainly on lectins, which are proteins that bind sugars. He likes to hike when he's not at the lab and is passionate about his plants and cats.

Lectins are ubiquitous proteins of non-immune origin that can reversibly and specifically interact with carbohydrates. They are involved in recognition events in various physiological and pathological processes like intercellular communication, adhesion, migration and host-pathogen interactions. Unlike antibodies, they are not product of immune response and do not possess any enzymatic activity. Lectins are commonly used for characterization of carbohydrate structures, for purification of glycoproteins and to specifically label the cell surface structures.

Photorhabdus luminescens is a naturally bioluminescent Gram-negative bacterium and an insect pathogen, which symbiotically lives in Heterorhabditidae nematodes. PluLec is a putative lectin from *Photorhabdus luminescens* and a homologue of PA-IL lectin, which is D-galactose specific, Ca^{2+} dependent, cytotoxic lectin from opportunistic pathogen *Pseudomonas*

aeruginosa, involved in facilitating infection in patients with compromised immunity.

This research is focused on structural-functional characterization of recombinant protein PluLec using various methods like isothermal titration calorimetry, hemagglutination, glycan array, analytical ultracentrifugation, toxicity tests made on insect models and protein X-ray and Neutron crystallography.

The study revealed that lectin crystallizes as a homotetramer with four binding sites for D-galactose (one per monomer). Shows specificity towards beta anomers of D-galactose. Preliminary results of toxicity test made on insect models shows clear negative effect on survival of insect. Obtained results of the structure and function of PluLec may reveal importance in the pathogenic or symbiotic stage of life.

This work was supported by the Czech Science Foundation (21-29622S) and we acknowledge the Biomolecular Interaction and Crystallization Core Facility and the Proteomics Core Facility, CEITEC, Masaryk University supported by the CIISB research infrastructure (project LM2018127 funded by MEYS CR) for their support with obtaining scientific data presented in this paper.

3'UTR-mediated regulatory partners in MYC mRNA across different cell lines

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My name is Sepideh M. Koubjari. I am a PhD student at Masaryk University and a member of Peter Lukavsky's research group at CEITEC MUNI, Brno. I have a Master's in Biophysics from Science and Research Azad University of Tehran and a Bachelor's in Cellular and Molecular Biology. During my master's studies, I worked as a Teaching Intern at Pandit Deendayal Petroleum University (PDPU), creating syllabus and teaching Introduction to Biophysics. My current research focuses on mRNPs, specifically proteins that interact with the 3'UTR of the MYC proto-oncogene.

The 3' untranslated region (UTR) of messenger RNAs (mRNAs) is critical in regulating various mRNA-dependent processes, including post-transcriptional regulation, which controls mRNA translation, stability, and localization. The 3' UTR of mRNAs is long with secondary and tertiary structures. This makes them attractive therapeutic targets. Our understanding of the range of regulatory partners operating through the 3'UTR is limited. However, it is known that post-transcriptional regulation can be influenced by microRNA (miRNA) machinery and signaling pathway proteins, which may act in a gene-, developmental stage-, tissue-, or cell type-specific manner. The 3'UTR of MYC proto-oncogene is highly structured with several RNA motifs, making MYC an attractive therapeutic target. MYC is a potent transcriptional regulator that drives tumor development, and its dysregulation is critical in oncogenesis. MYC expression is tightly regulated, with multiple mechanisms controlling its levels. Therefore, MYC is the first target of this study. The study

aims to identify the potential miRNA and RNA binding proteins (RBPs) responsible for regulating expression via its 3'UTR.

Our initial findings indicate that MYC 3'UTR significantly affects translation rates in both HeLa and HEK cells, with a more pronounced effect observed in HEK cells. Several miRNAs were selected based on their ability to target the MYC 3'UTR region and their higher expression rate in each cell line. Using the RNA pulldown technique in HEK-293 and HeLa cells overexpressing the MYC 3'UTR, RBPs that play a vital role in regulating and processing mRNA were enriched. Future work will extend the study to other cell lines and investigate different disease-related mRNAs to better understand mRNA regulation.

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Structural analysis of phosphorylated full length Tau protein fibrils without enhancers

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My name is Gytis Kučinskas, I am a 3rd year PhD student at Protein Structures and Dymanics group at CEITEC-Masaryk University supervised by Jozef Hritz. I am focusing on structures of recombinant Tau protein fibrils. I graduated in BSc Biochemistry at the Unversity of Essex in 2017. My research project was bioinformatics-based research in correlated mutation analysis of GPCR's group of GLP1-R proteins. Then I did my MSc in Drug Discovery and Pharmaceutical Science at the University of Nottigham. Over there I was focusing on coagulation factor XII alpha crystallization with small home-synthesised ligands and analysis using various assays. After graduation, I was working as a chemist and later as a scientist I in research and development group of recombinant proteins at the Thermo Fisher Scientific Baltics for 2 years. During my time in academia and industry I developed strong background in recombinant protein preparation and quality control. Moreover, I gained strong communicational and analytical set of skills that allows me to quickly react and adapt in fast pace dynamic working environment. Currently I am using structural biology methods such as Cryo-electron microscopy and atomic force microscopy for analysis of Tau protein fibrils. Recently I completed an internship at the University of Pittsburgh at the structural biology department where I gained knowledge in helical reconstruction of protein aggregates.

Neurodegenerative diseases such as Alzheimer's disease (AD) remain one of the challenges in modern-day medicine. Tauopathies are known as one of the causes of the progression of AD. Despite the understandable physiological progression of the disease, we are still lacking knowledge of mechanisms at the molecular level that could lead to the identification of potential drug targets with blockbuster therapy development.

Tau protein under normal physiological conditions is responsible for the stability of microtubules in neurons. Post-translational modifications such as phosphorylation or hyperphosphorylation of Tau lead to the formation of ordered aggregates known as tau fibrils that cause the loss of microtubule stability leading to neuronal cell apoptosis and progression of AD symptoms. Previous Cryo-EM-based studies identified two distinct Tau fibril conformations in the brains of patients with AD. (1) Moreover, heparin was identified as a Tau fibril formation-enhancing agent that generates four different types of fibrils. None of these conformations are identical to AD-causing structures. (2) Recently, a huge study with different types of constructs of full-length Tau (2N4R) was performed which revealed the fibrilization conditions, under which AD fold can be generated in Vitro. (3)

During our study, we managed to prepare hyperphosphorylated full length Tau filament without using any fibrilization-enhancing agent. We use this isoform because it is the longest and most abundantly found in the human brain. (4) Recombinant Tau40 was expressed and purified using a home-optimized scheme. Protein Kinase A (PKA) was used as a hyperphosphorylation agent. MALDI-MS and digestion with Trypsin allowed us to identify phosphorylation sites and quantify them. Fibrillization conditions were done based on previously reported results and results of other research. (3) With slight adjustments of agitation speed, we found that the best-growing conditions for hyperphosphorylated Tau40 protein are with phosphate buffers at 37 degrees Celsius with 800 rpm of agitation. Negative staining and atomic force microscopy techniques were used to identify fibrillar structures with helical features. Samples were prepared for Cryo-EM and micrographs were collected for analysis of atomic structure. CryoSparc is being applied for 2D classification and helical reconstruction. We are presenting intermediate results of a near-atomic model which is still in the progress.

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Dual binding mode of Dishevelled PDZ domain

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I am a PhD student in the group of Dr. Konstantinos Tripsianes at the Central European Institute of Technology, Brno, Czech Republic. Here at Tripsianes group, we study the biochemistry of Wnt signaling components (especially Dishevelled and Casein Kinase family) at the molecular level. My work aims at studying the conformational plasticity of the Dishevelled protein using several biophysical methods. I also run a science communication project called "Reason with Science" that aims to foster critical thinking and comprehension of the scientific method.

The Dishevelled (DVL) protein is the central hub of Wnt signal transduction. DVL function in Wnt signaling depends on its conformational plasticity. Indeed, the C-terminus of DVL is tucked away in its own PDZ pocket, acting as a switch between open and closed conformations that directs different biological outputs. Here we studied the molecular determinants of DVL closed state using biophysical methods. PDZ domains recognize a free carboxyl group in the interacting partners, either at the end of the linear sequence (terminal mode) or the side-chain of aspartate residues anywhere in the linear sequence (internal mode). The sequence of the DVL C-terminus contains both an internal and a terminal carboxyl group for the PDZ interaction. Despite being a distinct class within the PDZ interactome, dual-binding mode ligands need to be better characterized due to the limited availability of structural data. The crystal structure of the DVL PDZ domain with the DVL C-terminal peptide revealed that both binding modes are plausible. However, based on structural analysis, the two binding modes are indistinguishable. We, therefore, designed peptides that retain one or another mode and studied the PDZ interaction by Nuclear Magnetic Resonance (NMR) spectroscopy. By analyzing the exchange broadening profile of amide and methyl interacting

groups in four peptides (dual, terminal, internal, none), we show that: 1) each mode supports PDZ recognition, b) the terminal recognition mode is stronger, c) in the complex the C-terminal peptide exchanges between terminal and internal modes, with the terminal one being the dominant. Our results reveal yet another aspect of binding plasticity for this class of PDZ ligands.

Investigating the therapeutic potential of clinically-approved drugs for chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML) *in vitro*

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I am a PhD student in the Functional Genomics lab. My research primarily revolves around the field of precision medicine, where I aim to establish a solid foundation for my future career. As part of my research project, I have the opportunity to investigate the mechanisms of drug resistance and sensitivity, as well as explore treatment options for patients with chronic lymphocytic leukemia and acute myeloid leukemia.

High-throughput screening allows us to rapidly identify potent compounds that extend beyond the scope of their original function. Its integration alongside next-generation sequencing aims to improve personalized treatment plans with a particular interest in finding novel treatments for resistant patients. This research project focuses on identifying effective compounds targeting primary patient samples and cell lines of chronic lymphocytic and acute myeloid leukemia. Additionally, it aims to explore the molecular mechanisms underlying the effectiveness of these compounds in greater detail.

In order to accomplish the objectives of our research, we implemented a drug screening pipeline utilizing a selection of 859 FDA and EMA-certified compounds with broad chemical space. This drug library is automatically added to cells on 384-well plates with a programmed liquid handling system epMotion (Eppendorf). The incubation of cells lasts 72 hours, and then the cell viability in response to drugs is determined by a Cell-titer Glo assay. Data are

normalized and hierarchically clustered in heatmaps, revealing the most effective compounds for individual samples and their molecular subgroups. These top hits are further analyzed and validated to assess the reproducibility and the magnitude of the effect.

Our data indicate the overall high efficacy of DNA-damaging agents, proteasome and HDAC inhibitors in both AML and CLL samples. In the case of CLL, we have stratified the primary samples into different molecular subgroups considering the genetic mutations in *TP53, ATM* and their *IGHV* status. Although we have identified several compounds specific to individual patient samples, substantial differences were observed between the groups distinguished by their *IGHV* status. Currently, we are also incorporating additional primary samples with *NOTCH1* and *SF3B1* mutations to our drug screening pipeline.

We further investigated the efficacy of our library on venetoclax-resistant AML cell lines. In addition to previously stated compounds, we observed a high potency of a CDK inhibitor (Flavopiridol) and a sodium-potassium ATPase inhibitor (Digoxin). We also included an investigational MCL-1 inhibitor which has previously been shown to sensitize venetoclax-resistant samples and was proposed as a compensatory mechanism towards BCL-2 inhibition by venetoclax.

We are currently adding more primary samples, with the focus on samples from venetoclax-resistant AML patients. Our plan is to conduct more comprehensive analyses and enhance the translational potential of our research. Furthermore, we have planned additional investigations to identify the molecular mechanisms underlying the observed efficacy of the selected compounds.

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DNA Damage Altered the Fate Determination in Stem Cells

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My name is Ahmadreza Lagzian and I am in the third year of my study. I am from Iran and living in Brno for about two years and a half. On my current project, I am working on a Stem cell under the supervision of Vladimir Rotrekl at the Department of Biology, Faculty of Medicine. Our research group working on stem cells as a suitable model for some diseases such as DMD (Duchenne muscle dystrophy), and NBS and studying the function and molecular mechanism. I have got my Bachelors's and Master's in Iran at the University of Tehran in Biochemistry and Biophysics. I have published three papers during my bachelor's and master's.

DNA damage has been implicated in the modulation of stem cell fate determination. In this study, we explored the role of DNA damage mechanisms, in stem cell fate determination. It has been established that there are two main DNA repair pathways in stem cells, homologous Recombination (Error free) and Non-Homologous End Joining (Erro prone). HR is the preferred error-free repair mechanism in stem cells, compared to NHEJ. Additionally, DNA damage can originate from both spontaneous events within the cell and external environmental factors such as Irradiation.

We hypothesized that DNA damage could disrupt stem cell fate determination. Literature supports the existence of three germ layers—endoderm, ectoderm, and mesoderm—that give rise to specific organs and tissues. To assess the impact of DNA damage on fate determination, we selected specific markers for each germ layer: AFP for endoderm, Brachyury (T) for mesoderm, and Pax6 for ectoderm. Two stem cell lines, CCTL14 and CCTL14 down regulated NBS, were utilized in the study. CCTL14 cells underwent directed (cardiac) differentiation as well as spontaneous

differentiation, while the other cell line underwent spontaneous differentiation only.

Initially, we evaluated the effects of DNA damage without external irradiation and observed no significant changes in germ layer expression. Subsequently, cells were irradiated on day 3 and day 10, with CCTL14 cells receiving 3 Gy and the other cell line receiving 0.5 Gy. Furthermore, we employed Methoxyamin treatment as a base excision repair (BER) inhibitor to investigate the effect of DNA damage in both cell lines. Additionally, Glutathione, an ROS scavenger and antioxidant, was applied exclusively to the L14NBS cell line.

Following cell collection on day 10, we observed significant differences in germ layer expression compared to the control group in both cell lines. These findings suggest that DNA damage can indeed alter stem cell fate determination. Our study provides valuable insights into the impact of DNA damage on the differentiation potential of stem cells and underscores the importance of DNA repair mechanisms in maintaining cellular integrity.

Keywords: DNA damage, fate determination, stem cells, homologous recombination, non-homologous end joining, germ layers, irradiation, Methoxyamin, Glutathione, regenerative medicine.

Recognition of RNA Polymerase II C-terminal domain by RPRD2

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Kateřina Linhartová is a PhD student in the Structural Biology of Gene Regulation research group. Her research interests focus on the structural and biophysical characterisation of proteins and protein complexes involved in the regulation of transcription by RNA Polymerase II.

The largest subunit of human RNA Polymerase II (RNAPII) contains highly flexible C-terminal domain (CTD) that is composed of 52 heptapeptide repeats (first half of repeats with consensus sequence YSPTSPS and second half largely degenerated in sequence). Several CTDs canonical and non-canonical residues can be subjects of post-translational modifications. Tyrosine, threonine, and serine residues undergo dynamic phosphorylation/dephosphorylation resulting in specific phosphorylation patterns throughout different stages of transcription cycle. These phosphorylation patterns are recognized by various transcription and processing factors during the transcription cycle. Therefore, CTD plays an important role in the regulation of transcription and coupling of transcription to post-transcriptional processes such as mRNA processing.

One of the human transcription factors that recognizes phosphorylated RNAPII CTD is RPRD2, but its exact role in the transcription cycle is still poorly understood. In this study, we show that RPRD2 recognizes specifically pSer2 or pThr4 phosphorylated forms of CTD via its CTD-interacting domain (CID). The interaction of RPRD2 CID with pSer2 phosphorylated CTD is further enhanced by additional phosphorylation on pSer7. To provide mechanistic details of the interaction between RPRD2 CID

with pSer2,7 CTD or pThr4 CTD, we solved the solution structures of both complexes using NMR spectroscopy. RNAPII CTD pSer2 and pThr4 phosphorylation occur during the late elongation and termination in yeast and humans. RPRD2 preference for these phospho-marks shown in this study, and similarity at some aspects to yeast transcription termination factor Rtt103, suggests the possible involvement of RPRD2 in late elongation or transcription termination.

Sequencing of long non-coding RNAs in exosomes in patients with colorectal cancer

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Marie Madrzyk is a dedicated PhD student, currently in her third year of study in Molecular Medicine at the Prof. Slaby laboratory. Her passion for understanding the complexity of the human body at the molecular level has guided her research focus towards the exploration of long non-coding RNAs and exosomes as early biomarkers for cancer. Before starting her doctoral journey, Marie completed her Bachelor's and Master's degrees in Biochemistry. In addition to her academic pursuits, Marie has a healthy appetite for knowledge beyond the laboratory as she enjoys experimenting with molecular gastronomy in her kitchen– after all, isn't cooking just another form of chemistry experiment?

The prognosis of patients with colorectal cancer (CRC) depends mainly on the extent of the disease at the time of diagnosis; therefore, early detection is one of the main prerequisites for successful treatment. Current research shows that exosomal long non-coding RNAs (lncRNAs) are associated with cancer development. As lncRNAs are often tissue specific, their quantification in exosomes is proposed as a non-invasive method for early detection of CRC. In this study, we aimed to optimize a protocol for analyzing exosomal lncRNAs from blood serum of CRC patients as potential diagnostic biomarkers.

Exosomes were isolated by size exclusion chromatography from 150 µl of serum of CRC patients and healthy donors. Their quality and quantity were confirmed by electron microscopy and DLS analysis, and protein

markers were detected by Western blot. After RNA isolation, cDNA libraries were prepared from the samples and sequenced using NextSeq 550.

We successfully isolated exosomes and verified their properties by several different methods. Libraries were prepared from all samples despite the very low volume of starting material. Sequencing data confirmed the presence of both protein-coding (50%) and non-coding RNAs, which consisted mainly of lncRNAs (28.2%), pseudogenes (15.2%) and other RNA types (6.5%). Results also showed significantly altered levels of some lncRNAs, the expression of which was able to distinguish samples from CRC patients from healthy controls. Using GSEA analysis, we observed significantly enriched classes of genes related to DNA repair or cell cycle regulation.

Our pilot data suggest that lncRNAs represent a significant fraction of the RNA present in exosomes and their differential levels have the ability to distinguish CRC patients from healthy controls. The analysis of enriched genes also showed a significant representation of lncRNAs involved in cell cycle regulation and DNA repair, suggesting their possible involvement in carcinogenesis. However, the results need to be verified in a larger cohort of patients.

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The role of long non-coding RNAs in the microenvironmental interactions of malignant B cells

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I'm a fourth-year PhD student in the Microenvironment of immune cells lab at CEITEC (Mráz lab). In my PhD project, I'm focused on studying the role of lncRNAs in chronic lymphocytic leukemia and how these lncRNAs affect the microenvironmental cross talk of CLL cells. Outside of lab work, I like to play volleyball and read books. I find the research of non-coding RNAs very interesting and hope that in the future, we will be able to fully understand this 'dark matter' of our genome and use this knowledge to help patients across the globe.

Chronic lymphocytic leukemia (CLL) is a disease largely dependent on the interactions of malignant B lymphocytes with the components of the tissue microenvironment (TME). TME hubs located in the lymph nodes create a physical space for CLL cells to interact with stromal cells, T cells, and nurse-like cells. Circulating CLL cells homing to the lymph node niches are provided with stimuli activating B cell receptor (BCR) and critical co-stimulatory signals, mainly T cell-derived cytokines and interleukins, such as CD40L and IL-4. This complex TME cross talk regulates the activation of neoplastic B cells and contributes to the aggressiveness of the disease.

Research in recent years has established non-coding RNAs to represent an intricate regulatory layer of many cellular processes. It has been shown by us and others that microRNAs act as regulators of BCR signaling propensity in the lymph node microenvironment, however, the role of long non-coding RNAs (lncRNAs) in regulating and coordinating microenvironmental cross talk remains poorly understood.

To address this issue, we carried out RNA-seq profiling of lncRNAs differentially expressed pre- and post- treatment in patients undergoing therapy with idelalisib, a PI3K inhibitor, which impairs the ability of CLL cells to migrate and home to the lymph node microenvironmental niches. These results were cross-validated with RNA-seq profiling of lncRNAs differentially expressed in CLL cell populations emerging from the lymph node, which identified dozens of lncRNAs. We focused on the subset of lncRNA candidates likely to be involved in TME cross talk and selected one of these candidates for further investigation.

To understand the role of the candidate lncRNA in microenvironmental interactions, we stimulated primary CLL cells with stimuli mimicking the pro-survival and pro-proliferation signals CLL cells receive in the tissue microenvironment. Our data suggests that microenvironmental stimuli strongly affect lncRNA levels in a time-dependent manner. Interestingly, we observed strong repression of lncRNA levels in response to canonical microenvironmental signals, with the exception of interleukin 4 (IL-4) stimulation, which yielded increased lncRNA levels. BCR-mediated activation is critical for the normal function of B cells; therefore, we examined the effect of BCR ligation on the transcriptional dynamics of the candidate lncRNA. This revealed a pattern of tight regulation of lncRNA expression in response to the different stages of BCR-mediated activation of leukemic cells. Our data also shows that BCR inhibitors impair the BCR-mediated lncRNA upregulation, both *in vitro* and *in vivo*, supporting the importance of BCR-related signaling in the regulation of the lncRNA.

Furthermore, the candidate lncRNA was found to be highly expressed in leukemic cells in comparison to healthy B cells. Intriguingly, several CLL-derived cell lines diminished the lncRNA levels, which might hint at an advantage of lncRNA depletion in these aggressive CLL models. In order to probe the biological function of the candidate lncRNA in a cellular context, we engineered a CLL cell line overexpressing the lncRNA and performed RNA-seq profiling of differentially expressed genes in resting cell state. However, we didn't identify any significant pathway fingerprint on the transcriptome of the engineered cell line, nor did we detect any changes in protein phosphorylation kinetics. Therefore, we decided to focus on the relevant microenvironmental cues and test the importance of the lncRNA upon cell activation. Using a functional assay for monitoring intracellular calcium flux induced by IgM stimulation, we determined that the lncRNA impairs early-response BCR signaling propensity. Additionally, we explored the effect of T cell-derived IL-4 activation in the context of lncRNA overexpression and investigated the IL-4-mediated signaling axis dynamics. Following the phosphorylation kinetics of this signaling pathway, we determined that the

candidate lncRNA affects the level of IL-4-induced STAT6 phosphorylation status.

The mechanism by which the candidate lncRNA affects these cellular processes is not yet clear. However, we determined the lncRNA to be localized in the cytoplasm, which, taken together with the lack of transcriptional effect, points to the lncRNA's physiological relevance at the signal transduction level. The observed phenotypic changes upon cell activation also suggest that the lncRNA mediates its biological activity on the background of microenvironmentally activated cells, rather than cells in resting state. To elucidate the precise molecular mechanism of action, we are planning to utilize RNA pulldown to map the lncRNA protein interactome.

We identified a novel microenvironment-dependent lncRNA involved in facilitating the cross talk of CLL cells. However, further investigation is needed to understand its precise functional role in the context of microenvironment-dependent regulatory networks.

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Hexameric lectin from Photorhabdus laumondii

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I obtained a bachelor's degree in Biochemistry and a master's in Biomolecular chemistry at Masaryk University. During my studies, I did an internship at the European Molecular Biology Laboratory (Hinxton, United Kingdom), where I worked on the structural database of macromolecular complexes. Currently, I am a PhD candidate in Structural Biology at the Central European Institute of Technology, Masaryk University. My PhD projects are focused on the structural characterisation of proteins involved in cell–cell interaction called "lectins".

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

unique binding pocket, which significantly impacts the binding properties of the PLU1.

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CYTOKININ-INDUCED DIRIGENT13 GENE INVOLVES IN THE LIGNAN PRODUCTION AND MEDIATES PLANT RESPONSES TO THE STRESSES

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My name is Alesia Melnikava, I have a Master Degree in Molecular Genetics and currently a PhD candidate in Jan Hejatko's Research Group Functional Genomics and Proteomics of Plants. My sphere of interests is Plant signalling, Development and Stress adaptation.

Dirigent proteins mediate regio- and stereo-specific monolignol coupling during the lignans and lignins formation [1]. As a complex phenolic polymer, lignin enhances plant cell wall rigidity, hydrophobic properties and promotes minerals transport through the vascular bundles in plant [2], whereas the ubiquitous but structurally diverse lignans are involved in plant defense (antioxidants, biocides, etc.) [3]. Arabidopsis dirigent proteins (DIRs) represent a large and still unexplored gene family, consisting of 25 members. DIR5 and DIR6 are implicated in the (-)-pinoresinol biosynthesis [4], DIR10/ESB1 was shown to control Casparian strip formation in the root endodermis [5], and DIR12/DP1 is specifically expressed in seeds and involved in neolignans synthesis [6]. According to the phylogenetic analysis *DIR13* and *DIR14* are paralogues of *DIR5* and *DIR6*, but they lack the conserved residues necessary for (-)-pinoresinol formation [7].

Hereby, we found *DIR13* to be root-specific, upregulated by cytokinins and active since early stage of postembryonic growth. DIR13-mCherry fusion protein is localised in the root endodermis of the differentiation zone with a strong expression in the overlying endodermal cells of developing lateral roots. We tested different stresses on *35S:DIR13* lines. Thus, in the presence of NaCl, *35S:DIR13* lines showed better germination rate and primary root growth in comparison to Col-0 WT. Under drought stress conditions *35S:DIR13* lines exhibited higher wilting resistance and better recovery after plant rewatering. Our results indicate that *35S:DIR13* plants have higher ROS

production in the apoplast triggered by NaCl stress and more lateral roots growing under normal and saline stress conditions. HPLC-MS/MS analysis of phenolic compounds revealed more than 140 statistically significant compounds differently accumulated in the roots of Col-0 WT and *35S:DIR13* lines. 8 up-regulated and 2 down-regulated on *DIR13* OE lines were annotated as (neo)-lignans. These findings suggest a potential role of DIR13 in plant stress responses through lateral root growth promotion, ROS production and probably accumulation of (neo)-lignans.

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Integrative structural biology approaches to characterize roles of intrinsically disordered MAP2c and Sigma-A

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My name is Subhash Narasimhan, a PhD student currently conducting my research at Dr. Lukas Zidek's lab at CEITEC (Central European Institute of Technology). My primary focus is on the structural characterization of the mycobacterial Sigma-A transcription factor and its novel interaction partner, MoaB2. Additionally, I am involved in investigating the structural characterization of MAP2c regulatory partners, which include 14-3-3 proteins, serine/threonine kinases, tyrosine-directed kinases, and proline-directed kinases, and their role in microtubule interactions. Prior to my current position, I worked at CCMB (Centre for Cellular and Molecular Biology) in Hyderabad, India. During my time there, my research revolved around the structural characterization of the mycobacterial fatty acid adenylating ligase (FAAL) and the standardization of biochemical assay to study and uncover the interface of the FAAL linear chain lipid transfer onto the acyl carrier protein domain of the polyketide synthase involved in the production of virulent proteins. With a strong background in structural biology and a focus on understanding the molecular mechanisms underlying essential biological processes, I am committed to advancing knowledge in the field and making valuable contributions to the scientific community.

Intrinsically disordered proteins (IDPs) are a class of proteins that lack a fixed three-dimensional structure and instead exist in a dynamic ensemble of conformations. IDPs are involved in a wide range of biological processes, including signal transduction, transcriptional regulation, and cell division.

The sigma-A ($\boldsymbol{\sigma}^{\mathbf{A}}$) protein is a bacterial transcription factor that plays a vital role in the initiation of transcription by association with RNA polymerase

(RNAP) driving transcription initiation. Through an unbiased search for interacting partners of M. smegmatis **σ^A**, a number of proteins were discovered, with MoaB2 being the most prominent. MoaB2 is a member of a universally conserved protein family that is mainly involved in the Molybdenum incorporation pathway, a trace element in bacteria. Several approaches were used to validate and characterize the **σ^A**-MoaB2 interaction which demonstrated that the binding was specific and independent of RNAP, as other sigma factors did not show binding to MoaB2. The structural analysis of MoaB2 revealed that the unique, unstructured N-terminal domain of **σ^A** plays a critical role in the **σ^A**-MoaB2 interaction. Functional experiments demonstrated that MoaB2 inhibits **σ^A**-dependent transcription and increases the biological stability of **σ^A**. In summary, this study uncovers MoaB2 as a novel binding partner of mycobacterial **σ^A**, which has an alternate moonlighting function in prokaryotes.

Microtubule associated protein 2c (MAP2c), an intrisically dirodered protein is a juvenile splicing variant of the MAP2 gene product, assumed to regulate growth of dendrite in neurons of developing brain. The most important functional region conserved among MAPs is the microtubule binding domain (MTBD) , regulating dynamic instability of microtubules. MTBD of MAP2c exhibits a high sequence homology with Tau, but MAP2c does not form aggregates like those found in brains of patients suffering from the Alzheimer's disease. MAP2c, as a regulatory protein, is itself regulated by numerous post-translational modifications, including phosphorylations by several kinases, and by interactions with upstream regulatory proteins such as 14-3-3 and neurosteroids. Recent studies in our lab showed that 14-3-3ζ isoform competes with microtubules at the MTBD of unphosphorylated MAP2c. To better understand the transient structural features of MAP2c MTBD with microtubules, we performed cryo-EM trails of MAP2c incubated with microtubules polymerized invitro from pig brains. To address the problems with helical reconstructions of the data, we are exploring collecting additional data sets with the introduction of kinesins for better data reconstructon of the alpha and beta tubulin chains. Another scope of the project explores the phosphorylation by serine/thereonine kinase Protein kinase A (PKA) which phosphorylated MAP2c and Tau at different sites thus, highlighting a branching point in the MAP2c and Tau regulatory cascades. MAP2c phosphporylation by Serine/Threonine and Proline directed kinases were studied by **1H,15N-HSQC NMR spectra**. The native phophorylation of MAP2c in neuroblastoma cell extracts and the dephosphorylation kinetics of MAP2c phophorylated by Serine/Threonine and Proline directed kinases were studied in presence of neuroblastoma cell extracts. To understand the MAP2c structural features at near native conditions, we conducted in-cell NMR studies

on MAP2c. Our preliminary studies explored the unphosphorylated [**15N**]MAP2c and [**15N**]MAP2c-phosphorylated by PKA in HeLa cell lines. Further studies are planned to explore conformational behavior of MAP2c in neuroblastoma cell lines.

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Natural genetic variability in multistep phosphorelay as a tool for elucidating drought adaptation in Arabidopsis thaliana

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Katrina Leslie Nicolas is a PhD candidate in Life Sciences at CEITEC Masaryk University, supervised by doc. RNDr. Jan Hejátko. Her research focuses on investigating the role of multistep phosphorelay components in Arabidopsis thaliana's response to abiotic stress. Her aim is to gain a better understanding of the plant signaling pathways involved in stress response, which could contribute to the development of more resilient crops.

Developing climate-adaptable plant varieties through molecular breeding and high-throughput phenotyping is crucial for addressing the challenges posed by severe weather, diminishing arable lands, and a growing global population. Plants exhibit remarkable flexibility in response to changing climatic conditions, regulated by endogenous factors like plant hormones and external stimuli. In this study, we aimed to identify natural genetic variants of critical regulators that enhance integration of environmental and cytokinin signaling pathways, and evaluate their potential as targets for generating climate-adaptable crops. By leveraging the 1001 Genomes resource, we identified natural variations in three cytokinin-responsive histidine kinases (AHK2, AHK3, and AHK4/CRE1) in Arabidopsis. Accessions carrying single nucleotide polymorphisms (SNPs) near known protein function regions were selected for further investigation. Root-elongation experiments and

transcriptomic analysis of cytokinin signaling reporter genes were performed to investigate the impact of genetic diversity on the responsiveness of signaling pathways to cytokinins. Accessions with altered cytokinin sensitivity were identified, and root morphology analysis revealed reduced root apical meristem size in more responsive AHK variants. Ligand-binding assays indicated that the identified SNPs may affect the transduction of signals downstream rather than the binding-mediated activation of cytokinins. Detailed phenotypic analysis demonstrated the influence of SNPs in AHKs on cytokinin signaling and their effect on abiotic stress responses, such as drought. These findings provide insights into the existence of a mechanism that enables fine-tuning of stress responses by modulating hormonal signaling sensitivity. Our research has significant implications for synthetic biology approaches aimed at enhancing crop resistance to abiotic stresses.

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Amphipathic helices can sense negative membrane curvature

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Peter Pajtinka is a predoctoral researcher and a PhD student within the research group of Robert Vacha at the Centre of Structural Biology, CEITEC Masaryk University. With a background in computational biophysics, Peter specializes in studying the interactions between proteins and lipid membranes, focusing on curvature sensing and the selectivity of antimicrobial peptides. He uses a range of tools, from computational methods such as molecular dynamics to various imaging methods, to better understand these interactions and their role in biological systems. He hopes to advance our understanding of these interactions through his work, with potential applications in drug development and disease treatment. In addition to his research, Peter enjoys spending time outdoors hiking and climbing.

Proteins that sense and respond to membrane curvature are essential for biological processes such as vesicle trafficking, endocytosis, and cell migration. These proteins are able to localize to membranes with specific curvatures, allowing them to perform their functions with high precision. The most prominent class of curvature sensors - BAR domains - can sense either positive or negative membrane curvature, depending on the shape of their membrane-binding surface. Many short amphipathic helices (AHs) have also been shown to sense positive membrane curvature, but no AH with a preference for negative membrane curvature has been discovered yet. Using

a systematic computational approach, we derived AHs that preferentially localize to negatively curved membrane regions in both coarse-grained and atomistic simulations. In addition, we identified properties of AHs that are necessary for sensing positive or negative membrane curvature. Therefore, our results reveal a novel mechanism by which the amphipathic helices can contribute to the membrane localization of peripheral and transmembrane proteins.

Peptide Sensitivity on Membrane Curvature and Dependence on Lipid Unsaturation

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I am a PhD student at CEITEC in Robert Vacha's group. I study phospholipid membranes and their interactions with peptides using Molecular dynamics simulations. I completed my bachelor's and Master from India and currently exploring the world of Science residing in Brno. My not-so-academic life would include traveling, painting, cooking or just spending time in nature.

The biological membranes of living organisms are diverse in their geometrical and biophysical properties. Various cellular organelles like the Golgi apparatus, Endoplasmic reticulum, or even plasma membrane could exhibit a range of curvatures. These curvatures can have important functional roles such as the transportation of materials in and out of the cell and enhancing the cell's ability to interact with its environment. Several biological processes like cell signaling, membrane trafficking, membrane fusion, etc, could depend on how proteins or peptides sense this geometrical property and cascades these processes.

The studies of the theory of lipid packing defects are the most common explanation in terms of the sensing behavior of peptides. Hydrophobic lipid chain patches that are exposed to the solution from a membrane are known as packing defects. As the membrane's curvature increases, so do the number of flaws and their size. There are drawbacks to this approach, and we propose that peptide-membrane chemistry as well as the depth at which they are inserted determine how much curvature may be sensed.

The physical properties of the membrane, such as membrane fluidity and permeability, are determined by the saturation of lipid acyl chains. We are attempting to understand the relationship between the unsaturation of lipid chains in the membrane and the curvature-sensing mechanism of leucine-serine model peptides and their analogs. We explore the effects of unsaturation on the sensed curvature by peptides using coarse-grained

molecular dynamic simulations of buckled membranes, including PUFAs. We can gain insight into the induced curvature by the peptides and their relationship to the depth of peptide insertion within the membrane through pressure profile calculations from the flat membrane simulations.

CRISPR/Cas9 Technology as a Useful Tool in the Study of Chronic Lymphocytic Leukemia

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Helena Peschelová is a fifth-year PhD student in Life Sciences at The Faculty of Science, Masaryk University. She is a member of the Functional Genomics group at CEITEC Masaryk University, led by Dr. Michal Šmída. Her research project focuses on chronic lymphocytic leukemia, which is the most common type of leukemia in the Western world. Helena utilizes gene editing methods, specifically CRISPR/Cas9 technology, to introduce mutations found in leukemic patients into commercially available leukemic cell lines. The resulting cell models enable the research team to investigate the impact of these mutations on leukemic cells and search for potential therapeutic targets specific to patients who carry these mutations.

Chronic lymphocytic leukemia (CLL) is characterized by genetic heterogeneity and a variety of somatic mutations, the most frequent of which targeting *ATM, TP53, NOTCH1, MYD88* and *SF3B1* genes. A thorough exploration of these mutations could shed light on the disease etiology, or even lead to the discovery of potential novel drug targets. However, CLL cells extracted from patients do not proliferate *ex vivo* and thus preclude lengthy experiments, such as CRISPR/Cas9 screening.

The aim of this study was to generate stable knockout (*ATM, TP53*) and knock-in (*NOTCH1, SF3B1* and *MYD88*) CLL cell lines and subsequently use them to investigate unique vulnerabilities specific to these mutations.

Using CRISPR/Cas9 technology in HG3 and MEC1 cells, both CLL-derived cell lines, we generated monoclonal isogenic cell lines carrying disruptive mutations in *ATM* or *TP53*. We also used CRISPR/Cas9-based homology directed repair to obtain HG3 cells with the most recurrent mutations of *NOTCH1* (P2514fs), *SF3B1* (K700E) and *MYD88* (L265P). The knockout cell lines show a complete loss of ATM or p53 proteins, as well as abrogation of downstream signaling pathways, documented by impaired activation of p21. In addition, the *ATM* knockout cells demonstrate reduced phosphorylation of KAP1, a protein involved in DNA damage repair. The obtained heterozygous *SF3B1* knock-in cell line shows defects in splicing, as evidenced by promoted aberrant splicing of *DVL2*. Further functional validations of these cell lines are still ongoing. A selected *ATM* knockout cell line was then subjected to genome-wide CRISPR/Cas9 dropout screening to identify genes whose deletion is selectively lethal with the disruption of the *ATM* gene. The CRISPR/Cas9 dropout screen has revealed eleven genes that might be in synthetic lethal relationship with the introduced mutations. In particular, *ZNRD1*, a DNA-directed RNA polymerase I subunit that plays a role in cell proliferation, might be synthetic lethal to ATM. The validation of these eleven hits is currently ongoing.

Both *ATM* and *TP53* knockout cell lines were also used for *in vitro* and *in vivo* studies of the performance of anti-CD19 chimeric antigen receptor (CAR) T-cells in genetically defined classes of CLL. Studies of CAR T-cells showed different effectiveness at eradicating tumor cells *in vivo* depending on the driver mutation, with *TP53* mutations connected to inferior performance of CAR T-cells.

In summary, using CRISPR/Cas9 technology we were able to generate a panel of isogenic cell lines carrying mutations frequently recurring in CLL patients. These cell lines are indispensable for further in-depth studies of the mutations' impact on CLL therapy.

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Unraveling the process of thermoregulation during the seed development in Brassica napus under heat stress

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I am a fourth year PhD student in the research group "Hormonal crosstalk in Plant Development" led by Dr Helene Robert Boisivon. I am currently doing my research in finding out the mechanisms underlying the process of thermoregulation in the development of the Brassica napus seeds under heat stress. Our research aims in improving the crop production by understanding the basic science behind the plant developmental biology using genetics and molecular biology techniques.

Global warming and its effect on crop yield are among the most significant problems faced in the 21st century. Over the past decades, the gradual rise in global temperatures has reached above 1℃, affecting the yield of many of the important crops like wheat, rice, and maize. Studies on plant development under warm temperature conditions provide knowledge about the temperature's influence on crop yield. *Brassica napus* (rapeseed or canola) comes from the agronomically important plant family Brassicaceae and is the second most widely produced oilseed worldwide. This project studies the development of three *B. napus* cultivars, Westar, Topas, and DH12075, in three temperature regimes, 21℃, 28℃, and 34℃ in long-day conditions. Characterizing the thermomorphogenesis of *B. napus* grown in long-term heat stress conditions identified accelerated plant growth, reduced fertilization rate, and increased seed abortion rate. The accelerated and

defective embryo development and pre-harvest seed sprouting in plants grown under heat stress suggest a possible reduction in seed dormancy. We identified a reduced expression of Abscisic acid (ABA) biosynthetic genes and dormancy markers. However, the phenotypes were not reverted by external ABA applications. We hypothesized a link between high temperatures, accelerated embryo growth, and the mechano-sensing pathway during the early seed maturation phase under heat stress, which are studied by using transcriptomic analysis and measurements involving seed coat structure. Studies in this research area will pave the way towards producing thermotolerant varieties of *B. napus* with better crop yield.

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An unbiased analysis of interplay between adenosine methylation and editing

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PhD student in Stepanka Vanacova's laboratory of RNA quality control, where we focus on mechanisms of nuclear RNA surveillance and degradation, non-canonical RNA tailing and internal RNA modifications. My projects focus on the role of the non-canonical polyA polymerase Trf4 in RNA processing and transcription termination in Saccharomyces cerevisiae and on the role of the RNA m^{6}A demethylase FTO in human cells.

The precise and unambiguous detection and quantification of internal RNA modifications represents a critical step for understanding their physiological function. Only handful of marks can be detected by reverse transcription and sequencing, some of those thanks to the additional chemical conversions of isolated RNAs. Detection and quantification of m^{6}A, m^{6}A$_m$ and m^{1}A modifications still remains one of the biggest challenges in the field. Second intriguing and timely question that remains to be addressed is the extent to which individual marks are co-regulated or potentially can affect each other. Here we present a study where we detect and quantify several key mRNA marks in human total RNA and mRNA. We show that the adenosine demethylases FTO and ALKBH5 primarily targets different modifications, thus specifying their targets and potential RNA substrates in HEK293T cells. Interestingly, upregulation of both FTO and ALKBH5 is accompanied by an increase in inosine level in overall mRNA, bringing up the role of innate immune response during protein overexpression in cells.

Exploring patterns in clinical, biological, and molecular data of leukemia patients with CLLue

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Tomáš Reigl is a PhD student in Life Sciences at CEITEC MU. His background is in Mathematical Biology (BSc) and Bioinformatics (MSc). Currently, he is mainly focused on developing bioinformatic tools with user interfaces. Within his PhD project topic 'High throughput sequencing data analysis of IG/TR rearranged genes in leukemia clinical research', he has been involved in the development of several software projects, e.g.: LYNX (LYmphoid NeXt-Generation Sequencing) analytical software, CLLue, ARResT/Interrogate, ARResT/Subsets aka Encyclopedia of CLL Subsets.

LYNX is a tailor-made bioinformatic tool for a new custom-designed capture-based NGS panel. With this tool, a user can analyze molecular markers, such as gene mutations, copy number variations, antigen receptor rearrangements, and translocations in the most common lymphoid malignancies.

CLLue is an interactive web-based tool that allows users to perform a visual exploratory data analysis, identification of important and significant features in given groups based on statistical tests, and de novo clustering on their data.

ARResT/Interrogate is a web-based, interactive application for immunoprofiling IG/TR sequence data, both NGS and Sanger sequencing. It was designed to process, analyze, organize, and filter large amounts of data by numerous criteria and their combinations based on user-provided arbitrary metadata and present them in several interactive visualizations.

The Encyclopedia of CLL Subsets is a publicly available online tool that facilitates access to the latest research findings on stereotyped CLL subsets, i.e., the homogeneous groups of CLL patients characterized by identical or highly similar antigen receptors in an otherwise heterogeneous disease entity. It also enables the analysis of individual patient data and result interpretation.

In the age of modern medicine and data science, it is possible to collect a vast amount of biological and molecular data about a patient from a single sample. By combining this information with a case history, we can explore patterns of clinico-biological features in the context of other patients with the same diagnosis. Finding these patterns could help separate patients into groups with significantly different characteristics and, e.g., potentially different responses to treatment. We have searched for such patterns in a local dataset of one hundred patients with chronic lymphocytic leukemia (CLL).

We have selected a dataset of one hundred CLL patients, each with two samples collected at two different time points - diagnosis and a follow-up after the median of 36 months. The data were curated, homogenized, merged, and transformed into a single table format. The final table was analyzed using CLLue, an interactive bioinformatic tool for data exploration, segregation, and important variables selection. The data were analyzed visually during exploratory data analysis (EDA) and computationally using statistical tests, machine learning and clustering.

CLLue provided interactive tools for the initial visual exploration of the dataset, which helped us filter out outliers and highly correlated values, as well as an expected number of final clusters. Using several sets of pre-selected variables in the dataset, we performed multiple clustering methods. This approach produced various clustering results, which were examined for variables that significantly define and distinguish the clusters. The most significant ($p\text{-value} < 10^{-8}$) and important (XGBoost Importance Gain > 0.1) variables were: absolute lymphocyte count (ALC) increase per month [rapid/slow], CLL activity [fast/slow/indolent], treatment after time point 2 (TP2)

[yes/no] and ALC in TP2, the time between TP1 and TP2 [in months]. Final clustering was performed only on the significant variables and produced three final clusters of possible clinical relevance.

We have demonstrated that the CLLue can provide users with a deeper understanding of a given dataset and create clusters of related data. However, the following data interpretation needs to be performed in the context of clinical data to prevent results from misinterpretation. Users of CLLue are currently also expected to have a minimum knowledge of statistical methods to ensure the appropriate tests are implemented. Therefore, the next phase of further development will focus on dynamic safety checks and warnings for users in the user interface of CLLue.

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Unravelling the molecular function of CDM1 zinc-finger protein in meiotic progression in *Arabidopsis thaliana*

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I'm Surendra Saddala, a doctoral candidate in Dr. Karel Riha's at CEITEC-MU. We focus on understanding plant meiosis using the model plant species Arabidopsis thaliana. I'm studying the function of an RNA-binding protein during meiosis progression and exit for my dissertation. To achieve this, I employed molecular cloning, reporter line creation, RNA Immuno Precipitation-Sequencing (RIP-Seq), protein expression and purification in bacterial systems, Western blot analysis of proteins, and several other methods. During my doctoral research, CEITEC-MU provided me with an excellent opportunity to access state-of-the-art core facilities that allowed me to understand plant reproduction better. Contact me on Twitter to learn more about RNA-binding proteins in plant meiosis at @surensrasaddala.

Plant meiosis, in contrast to animals, is followed by several mitotic divisions to produce functional gametes. The transition from meiosis to post meiotic development is poorly understood. We discovered that in *Arabidopsis*, this transition during male gametogenesis is governed by unknown mechanism that involves *SUPPRESSOR WITH MORPHOGENETIC EFFECTS ON GENITALIA7 (SMG7), THREE DIVISION MUTANT1 (TDM1)* genes. Mutations of these genes leads to third meiotic division and fail to produce microspores, which results in male sterility. To decipher this mechanism, we performed a forward genetic screen to identify genes that

rescue fertility of *smg7* mutants. We found two recessive and one dominant mutations in a gene coding for *CALLOSE DEFECTIVE- MICROSPORE1 (CDM1)*. *CDM1* is a transcription factor required for the formation and dissolution of callose in male meiosis and secondary cell wall formation. In my Ph.D. project, I aim at unraveling the molecular function of CDM1 during meiosis.

We confirmed through both genetic association studies and complementation experiments that mutations in *CDM1* restore fertility and microspore formation of *smg7* and *tdm1* mutants. *CDM1* encodes 308 amino acids protein containing two Tandem Zinc Finger (TZF) motifs separated by a linker at the C-terminus. Reporter lines lacking TZF motifs could not complement the null mutation, demonstrating that TZF motifs are crucial for molecular functions of CDM1. CDM1-GFP reporter lines showed that CDM1 forms distinct cytoplasmic foci specifically in cells undergoing meiosis. Ectopic co-expression analysis in leaf protoplasts revealed that CDM1 co-localizes with DCP1, a marker of P-bodies, which are the hubs for RNA processing. Further in-vitro experiments indicated that, the CDM1-GFP has the propensity to form Liquid-Liquid Phase Separation (LLPS) condensates in the presence of RNA. It implies a role of CDM1 in RNA metabolism during meiosis. Localization of CDM1 to P-bodies and presence of TZF motifs indicate direct binding of CDM1 to RNA and its metabolism. Our further research is aimed at identification of transcripts that associate with CDM1 and subsequent elucidation of CDM1 function in meiosis exit.

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HSP101 and HSBP mediate gamete and embryo development during thermal adaptation

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My name is Juan Francisco Sánchez López, I am a PhD candidate in the research group led by Dr. Hélène Robert Boisivon. I focus my research on how high temperatures affect embryo and seed development. In the last decades we have suffered an increase in global temperatures which has a negative impact on crop production. In order to elucidate how plants cope with high temperatures, we mimic some natural stress conditions in the lab to study the long term adaptation to high temperatures.

Over the past few decades, we have observed global repercussions of climate change, including a steady increase in average global temperatures and extreme temperature occurrences. As a result, the production of temperate crops has decreased, primarily due to high temperatures and droughts, and this trend is expected to worsen in the years to come. When it comes to plants, the reproductive phase is a sensitive developmental stage that is particularly susceptible to high temperatures, which can negatively impact heat-sensitive processes such as pollen development. A complex response to high temperatures involves transcription factors, chaperone proteins, and phytohormone production.

HSP101 is one of the most important chaperones in *Arabidopsis thaliana*. It plays a key role in thermomemory and adaptation to temperature stress (Hong & Vierling, 2001). It also regulates flowering under non-stress conditions like other chaperone networks such as HSP90 and HSP70 (Qin *et al.*, 2021). The *hot1-3* mutant is a knocked-out mutant of HSP101, which has been widely used in plant research as a positive control due to its hypersensitivity to high temperatures.

The Heat Shock Binding Protein (HSBP) is the master negative regulator of the Heat Shock Factors (HSF), which are the most important transcription factors regulating the heat shock response (Rana *et al.*, 2012). Previous reports showed that mutation in *HSBP* reduced the fertility of plants, but the mechanism behind it remained unclear (Hsu *et al.*, 2010).

In our experiments, we allowed the different mutants to adapt to different high-temperature scenarios to study how plants adapt to long-term exposure to stress conditions. We showed that both HSP101 and HSBP are essential elements mediating pollen, ovule, and embryo development in Arabidopsis at high temperatures. Ovule development is significantly affected in the *hsbp-2* mutant mutants, while pollen is severely disrupted in the *hot1-3* mutant when grown at high temperatures. The embryo development of the *hsbp-2* mutant is impacted at control temperatures and dramatically affected at high temperatures.

Our results elucidate new aspects of plant adaptation to stress. For the first time, we showed that ovule development is affected by high temperatures in *Arabidopsis thaliana*. High temperatures also cause the acceleration of embryo development, leading to abnormalities during embryogenesis and, eventually, abortion.

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The intracellular auxin homeostasis regulators PIN5 and PIN8 have a divergent membrane topology in *Arabidopsis thaliana* root cells.

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I am an early career scientist who has 4 years of teaching experience as a lecturer and 5 years of research experience in the field of plant developmental and cell biology, post-harvest physiology, and crop protection. I am also good in leadership. I am passionate to transform and use these experiences to solve different problems in crop production. I love to implement the RNAi approach to develop transgene-free and sustainable methods to control plant fungal diseases and parasitic weeds. I am highly interested to share and transfer my knowledge and experiences through teaching and collaboration in research.

PIN proteins establish the auxin concentration gradient, which coordinates plant growth. PIN1-4 and 7 localized at the plasma membrane (PM) and facilitate polar auxin transport while the endoplasmic reticulum (ER) localized PIN5 and PIN8 maintain the intracellular auxin homeostasis. Although an antagonistic activity of PIN5 and PIN8 proteins in regulating the intracellular auxin homeostasis and other developmental events have been reported, how the two proteins, which localize at the same intracellular compartment, antagonize each other remains unclear. Combining immunolocalization, pH-dependent fluorescent quenching, and topology prediction programs, we mapped the membrane topology of PIN5 and PIN8 in *Arabidopsis thaliana* root cells. Our results indicate that, except for the similarities in the orientation of the N-terminus, PIN5 and PIN8 have an opposite orientation of the central hydrophilic loop and the C-terminus, as well

as an unequal number of transmembrane domains (TMDs). PIN8 has ten TMDs with groups of five alpha-helices separated by the central hydrophilic loop (HL) residing in the ER lumen, and its N- and C-terminals are positioned in the cytoplasm. However, the topology of PIN5 comprises nine TMDs. Its N-terminal end and the central HL face the cytoplasm while its C-terminus resides in the ER lumen. Overall, the divergent membrane topology of PIN5 and PIN8 reflects different and often mutually opposing activities of these intracellular auxin homeostasis regulators.

Keywords: Arabidopsis thaliana, auxin homeostasis, auxin transport, endoplasmic reticulum, membrane topology, PIN auxin efflux carriers.

Staufen1 interaction with target 3' UTR and influence of SSM and other domains on Staufen1 oligomeric state

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Huma Shakoor is a highly dedicated PhD student in life science, currently conducting research at CEITEC, MU, Brno. Her research focuses on exploring the interaction between Staufen and 3´ UTRs. With a long-term goal of becoming an expert in Structural and Molecular Biology, Huma aims to contribute to the development of this field both internationally and in her home country.

Human Staufen1 (hStau1) is a double-stranded RNA binding protein involved in various biological processes such as mRNA transport and localization, Staufen mediated mRNA decay (SMD) as well as regulation of mRNA stability and translational efficiency. Staufen1 consists of several domains including dsRNA binding domains (dsRBD2, dsRBD3, dsRBD4, dsRBD5), a Staufen Swapping Motif (SSM) and a Tubulin Binding Domain (TBD). The SSM domain is essential for the dimerization of Staufen. Staufen can bind to specific 3'UTRs by its RNA binding domains (RBDs) dsRBD3 and dsRBD4 and stimulates mRNA degradation by dimerizing with another Staufen protein. In order to understand SMD, our study aimed to investigate how double-stranded RNA binding proteins accomplish specificity for Staufen dimerization. Therefore, we focused our experiments on evaluation of hStau1 dimerization and its binding to RNA. The impact of SSM deletion on Staufen dimerization and RNA binding was assessed by Small Angle X-ray Scattering (SAXS), Analytical Ultracentrifugation (AUC) and Fluorescence Anisotropy (FA). Moreover, the stoichiometry of Staufen RNA interaction was analyzed by Isothermal titration calorimetry (ITC) and Size exclusion chromatography column in-line with multi-angle light scattering (SEC-MALS). SAXS data suggests that hStau1 is characterized by several elongated tertiary structures. According to AUC results, we observed the concentration dependent-dimerization in full length Staufen1, RBD3-End, RBD4-End. However, we did not detect dimers in full length Staufen1ΔSSM, RBD3-EndΔSSM, RBD4-EndΔSSM indicating that the deletion of SSM dramatically reduces

dimerization. Interestingly, FA data demonstrate that SSM deletion does not significantly affect Staufen RNA binding. These findings suggest that SSM is involved in Staufen dimerization but not in Staufen-RNA interactions. Our ITC and SEC-MALS results confirmed that the interaction between Staufen and RNA follows a 2:1 stoichiometry. Overall, our findings provide insights into the structural and functional features of hStau1 and its derivatives, which may have important implications for understanding the protein's role in various biological processes.

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Exploring the Interplay between ADAR1, MAVS, and PKR in Innate Immune Responses

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Ketty Sinigaglia, is a Doctoral student in the RNA and Immunity group led by Professor Mary O'Connell. After completing her Master Degree in Molecular Biology at the University of Milano Bicocca, she decided to take the chance to move to Brno, at CEITEC, an innovative and young research center and begin an exciting project in the epitranscriptomic field. She is going to conclude her studies in the next semester and to start a new thrilling adventure in Milan in the field of Clinical Research.

ADAR enzymes emerge as master architects, orchestrating the deamination of adenosine into inosine within the dsRNA. Their profound impact extends far beyond mere chemical transformations, enabling cells to discern endogenous RNA from exogenous RNA, preserving immune homeostasis. The accumulation of endogenous unedited ADAR1 RNA substrates triggers a cascade of events, culminating in the production of type I IFN, through the anti-viral MDA5/MAVS pathway. Loss-of-function mutations in ADAR1 result in Aicardi-Goutières Syndrome (AGS), a congenital encephalopathy resembling viral infection. In murine models, Adar1 deficiency leads to embryonic lethality by E12.5 with aberrant immune response, failed hematopoiesis and widespread apoptosis. However, concurrent deletion of Mavs or Mda5 allows the mice to survive for a few days after birth.

We investigated the intricate relationship between ADAR1, MAVS, and the dsRNA-dependent protein kinase PKR, (encoded by Eif2ak2 gene), in the innate immune response. We observed that the apoptosis detected in the small intestine of *Adar1,Mavs* mice at P14, only appeared at P8 in the proximal intestine and progressed throughout the organ. Remarkably, our findings revealed that deletion of Eif2ak2 partially rescued the mortality by delaying the intestinal apoptosis and preventing the interferon stimulating genes (ISGs) and Integrated stress response (ISRs) increasing.

Pkr activation is prevented in the enzymatically inactive Adar1 embryos suggesting that Adar1 and Pkr could compete for the same dsRNA substrate and that the presence of the inactive Adar1 is sufficient to avoid Pkr pathway activation.

We also confirmed a regulatory interaction between PKR and ADAR1 that inhibits the phosphorylation of the PKR kinase domain and activation of PKR in A549 cells. Using specific point mutations in the third dsRNA binding domain (RBDIII) of ADAR1, we showed that ADAR1 suppresses the activation of PKR in an editing-independent manner.

In summary, our study provides novel insights into the complex interplay between ADAR1, MAVS, and PKR in regulating innate immune responses. This may pave the way for developing targeted interventions for related disorders and shed light on the broader role of ADAR1 in immune modulation.

Bioinformatic Pipeline for Comprehensive Analysis of Small RNA-seq Data

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Karolína Trachtová is a PhD student in Life Sciences, focusing on bioinformatics and the analysis of sequencing data to advance our understanding of RNA and its cellular functions. Her research is primarily centered around non-coding RNAs, such as microRNAs, tRNA fragments, and long non-coding RNAs. She possesses the expertise to analyze data from various sequencing methods, including cutting-edge spatial transcriptomics. Proficient in both R and Python programming languages, Karolína effectively applies her skills and broad statistical knowledge to extract meaningful insights from complex datasets. With her multidisciplinary skills, she is dedicated to contributing to the field of molecular biology and advancing our knowledge of RNA biology.

Next-generation sequencing (NGS) is a powerful method that enables massive parallel sequencing of millions of DNA or RNA fragments. However, despite its widespread use, there is still a need for comprehensive bioinformatical approaches for NGS data analysis, particularly in the field of small RNA research. Accurate identification and quantification of the full spectrum of small RNA classes, including snoRNA, snRNA, piRNA, and isomiRs, is critical for obtaining reliable results. Unfortunately, most existing pipelines only focus on microRNA and ignore other important small RNA classes.

To address this issue, we have developed a novel bioinformatic pipeline for the accurate quantification of various small RNA classes. Our

pipeline consists of stand-alone modules, each of which is dedicated to a specific part of the sequencing data analysis, including quality control, pre-processing, RNA quantification, and differential expression analysis. The most crucial is the RNA quantification module, where a successive number of mapping rounds ensure accurate quantification of all different small non-coding RNAs. To achieve this, we have created a custom Python tool that counts reads assigned to different small RNAs while addressing the issue of multi-loci RNAs (such as piRNA) and overlapping RNA annotations.

To aid in the interpretation of results, each module generates a comprehensive PDF/HTML report that includes tables, plots, and explanations. The report guides users in further exploring various small RNA expression levels. Moreover, we have developed an interactive application implemented in Shiny that allows real-time visualization of differential expression results. This application enables users to easily modify the content and appearance of popular plots such as heatmaps, principal component analysis (PCA), and volcano plots, making it ideal for publication-ready figures.

Overall, our novel bioinformatic pipeline offers a comprehensive approach to the analysis of small RNA sequencing data. Our pipeline is flexible, scalable, and user-friendly, providing researchers with a valuable tool for exploring the complex landscape of small RNAs.

Core Facility Bioinformatics of CEITEC Masaryk University is gratefully acknowledged for the obtaining of the scientific data presented here.

In-situ cryo-electron tomography of Enterovirus replication

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Zuzana Trebichalská is a 3rd year PhD candidate at Plevka Lab, Structural virology, CEITEC MU. She is investigating the replication cycles of enteroviruses with the use of cryogenic electron microscopy. To share her results and interest, she has not only attended multiple international conferences, but also has given a talk at the science popularization show Science slam. When not working by the microscopes, she can be found hiking the various trails in Czechia or Slovakia, or occasionally bouldering.

Enteroviruses, a large group of non-enveloped picornaviruses, cause a range of human diseases, from the common cold to poliomyelitis. The molecular mechanisms of enterovirus replication and virion assembly are not well understood.

Here, we present the cryo-FIB milling and cryo-electron tomography of Echovirus 18-infected cells to characterize the late stages of enterovirus infection *in situ*. We describe rearrangements of cellular membranes that enable the formation of viral replication factories. Furthermore, we have identified different virus particle assembly intermediates – capsid segments, open and complete capsids, and progeny virions. Some of the virus particles and assembly intermediates were localized in the cytoplasm, whereas others were bound to membranes. We have employed template matching using the structures of the whole virus capsid and a capsid missing three pentamers to identify the complete genome-containing and empty virus particles and virus assembly intermediates, respectively. The particles have been further analysed by subtomogram averaging and classification.

Our results provide insight into the virus arrangement during the late stages of the replication cycle of enteroviruses. Additionally, the employed subtomogram averaging pipeline presents a further analysis of viruses and large molecular complexes *in situ*.

We acknowledge the Cryo-electron microscopy and Tomography Core Facility of CEITEC MU for their support in data collection and analysis.

Automated Analysis of Grain Spikes in Greenhouse Images Using deep learning models

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As an Image Analyst at PSI, I utilize my expertise to evaluate intricate Greenhouse image data. Prior to this role, I worked as a Research Specialist for three years at the Plant Sciences Core Facility-CEITEC, refining my research abilities and collaborating with diverse teams.

Automated analysis of small and optically variable plant organs, such as grain spikes, is highly demanded in quantitative plant science and breeding. Previous works primarily focused on detecting prominently visible spikes emerging on the top of the grain plants growing in field conditions. However, accurate and automated analysis of all fully and partially visible spikes in greenhouse images renders a more challenging task, which was rarely addressed in the past. A particular difficulty for image analysis is represented by leaf-covered, occluded but also matured spikes of bushy crop cult ivars that can hardly be differentiated from the remaining plant biomass. To address the challenge of automated analysis of arbitrary spike phenotypes in different grain crops and optical setups, we performed a comparative investigation of six neural network methods for pattern detection and segmentation in RGB images, including five deep and one shallow neural network.

Our study showed that DNN models' performance on a relatively modest set of ground truth images depends on the optical spike appearance (phenotype) and the spatial spike location within the plant in different crop cultivars. Detection DNNs showed an accurate and robust performance crossover of different crop cultivars, including wheat, barley and rye plants. From this observation, we conclude that DNNs trained on a particular s et of plant images can generally be expected to show comparable performance by spike detection in other phenotypically similar crop cultivars. For pixel-wise

spike segmentation, DNN models, such as DeepLabv3+ and U-Net, performed better than the conventional shallow ANN. Otherwise, DNNs showed accurate segmentation results by applying images of identical cultivar phenotypes as in the training set. A particular challenge for DNN segmentation models seems to represent pixels on the spike boundary, as they exhibit substantial variations in color and neighborhood properties, depending on the type of grain crops (e.g., grain with spikes emerging on the top of the plant vs. bushy plant phenotype; spike color, texture, size, shape); and scene illumination. From the results of this study, we conclude that a considerably larger set of different plant and spike phenotypes is required to achieve significantly more accurate segmentation of spikes. Using DNN models. On the other hand, tested segmentation models (U-Net, DeepLabv3+) appear suitable for accurate spike segmentation in large image sets after training on a relatively modest amount of ground truth images. This makes them suitable tools, especially when processing large amounts of phenotypically similar images is the primary goal.

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Carpe pili!
Hunting strategy of phage JBD30 revealed by combination of cryo-electron and fluorescent microscopy

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Lucie is doing her PhD in a group of Structural Virology of Dr. Pavel Plevka at CEITEC MUNI. She foccuses on bacteriophages. Using the recent advances of cryo-electron and fluorescence microscopy she is trying to reveal the mechanisms of phage - bacterium interations at time resolved molecular level. In 2023 she completed her internship in Joana Azeredo group at University do Minho, learning how to prepare genetically modified phages via the yeast cell based genome engineering platform. Besides the wet lab work, she enjoys hiking and sleeping under the stars.

Increasing numbers of infections caused by multi-drug resistant bacteria have renewed the interest in phage therapy. However, the exact mechanisms of phage – bacterium interaction are not known for the most of them. Here, we present the virion structure and infection strategy of *Siphoviridae* bacteriophage JBD30 revealed by the combination of cryo-electron and fluorescent microscopy.

Bacteriophage JBD30 uses its tail fibres for recognising *P. aeruginosa* pili type IV. After the attachment to pili, the virion either diffuses along it or is pulled by pili retraction towards the cellular surface, where it irreversibly binds by the tripod complex of receptor binding protein trimers. Afterwards, the phage punctures the outer cellular membrane and degrades the peptidoglycan

layer using the enzymatically active domains of receptor binding protein and tape measure protein C-terminal trimeric α-helical coiled coil domain.

Bioinformatic analysis of tape measure protein showed that its N-terminal part is composed of three domains: hydrophobic transmembrane domain I (residues 57–79), cytoplasmic domain (residues 80–384) and hydrophobic transmembrane domain II (residues 385–409). Furthermore, the estimated size of these domains corresponds to the thickness of *P. aeruginosa* cellular membranes. We assume that the N-terminal part of the tape measure protein might form a channel spanning the whole cell wall facilitating DNA transition from the virion capsid into the host cytoplasm. New phage progeny is released approximately 80 minutes post ejection of phage DNA into the host cell.

The combination of cryo-electron microscopy analysis techniques, cryo-electron tomography and fluorescent microscopy allowed us to propose the mechanism of key stages of phage infection and describe it at time resolved molecular level.

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Uncovering Retroelement Activity in Hematological Malignancies

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I have completed Master of Science in Biology, with specializing in genetics from Belarusian State University, Biological faculty in 2015. My graduation work was focused on research of the structural diversity of RNA products of RUNX1T1- part of the fusion oncogene RUNX1/RUNX1T1. It was defended with grade A (10/10). My previous individual 2 years' study at the Belarusian Research Center for Pediatric Oncology, Hematology and Immunology was focused on detection and analysis of genetic alterations of several tens of oncomarkers (ALL and AML patients in particular), minimal residual disease (MRD) monitoring, clinical research. From 2017, I am a PhD candidate at Masaryk University and CEITEC, my thesis work is about activity of transposable elements in haematological malignancies. I am interested in human genetics research, clinical analysis of leukemia patients, biomarkers discovering, searching and understanding of mutations in cancer associated genes using bioinformatics analysis.

The majority of transposons in the human genome consist of retroelements (RE), which are categorized into various families. Long interspersed element-1s (LINE-1; L1) are responsible for ongoing RE retrotransposition through RNA-mediated mechanisms. Alu, a subgroup of short interspersed elements, is nonautonomous and relies on L1-encoded proteins for mobilization. The potentially harmful impact of REs on genome

stability is prevented by suppressing RE expression in normal cells. Due to malfunction in cancer, REs escape genomic DNA methylation and transcriptional repression, and generate new REs insertions that can affect tumor initiation and progression.

We aimed to explore RE activity in chronic lymphocytic leukemia (CLL) and myelodysplastic syndrome (MDS), focusing on *TP53* gene defects and demethylation therapy as they may affect RE expression. To identify tumor-specific RE insertions, we adopted an NGS protocol for detecting Alu-Ya5, Alu-Yb8, or L1-HS families (the most active in humans) and their adjacent genomic regions. In total, 82 samples from 31 patients were analyzed (17 CLL, 14 MDS; 65 tumor DNAs for both diagnoses, 17 normal DNAs for CLL). Besides, we analyzed four cell lines derived from CLL: HG3, an original clone and two clones with *TP53* inactivated (*TP53*mut) via CRISPR/Cas9, and MEC1. First, the method sensitivity was evaluated, revealing the 1% detection threshold for the proportion of cells with specific RE insertion. Following this result, we identified three candidates for new tumor-specific RE insertions in the tested cohort, which will be validated using PCR.

To observe the RE transcriptional activity in CLL patient samples and cell lines, RNA-seq data analysis using the TElocal tool was performed. The results show a lower transposon expression in CLL patient samples with *TP53*mut expansion and in *TP53*mut cell lines in comparison with stable CLL patient samples without *TP53*mut and the *TP53*-gene-wild-type cell line. The most differentially expressed families were represented by older RE families, likely due to the co-expression effect of nearby coding regions.

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Deciphering the global proliferative arrest: an elusive link between plant reproduction and longevity

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Darya Volkava is a PhD candidate in the research group of Dr. Karel Riha. Prior to joining CEITEC MU, she studied at Belarussian State University in Minsk, Belarus, where she obtained biology specialist degree (equivalent of B.Sc. + M.Sc.) in 2017. Darya is interested in how plants coordinate their growth and reproductive efforts with environment they live in.

Proliferation of meristems defines both the final shape of body and longevity of plant. When meristems proliferation ceases, plant stops its growth and eventually dies. In many plant species, including annual crops such as rapeseed, rice, maize, as well as in model *Arabidopsis thaliana*, inflorescence meristem (IM) activity and, hence, plant longevity are coupled with reproduction. Once plant produces a predetermined number of seeds, it inhibits activity of all IMs and stops forming new flowers. This phenomenon is termed global proliferative arrest (GPA) and indicates existence of a systemic signalling mechanism that measures number of produced seeds and communicates it to meristems. GPA has important implications for crop yield, but little is known about its molecular underpinning.

We attempt to dissect this process by quantitative imaging of inflorescence meristem at different stages of plant development using a unique technology that enables 3D reconstitution of meristems from light sheet microscopy data. We focus on changes in hormonal signalling and proliferative activity in IM during its progression towards GPA and propose a model for meristem arrest.

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mRNA N6-adenosine methylation (m6A) integrates multilevel auxin response and ground tissue development

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I am a PhD student in the field of plant genomics, specializing in the study of RNA modifications and their role in plant development and hormonal pathways. I work in the RNA Processing Group at the Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany of the Czech Academy of Sciences, in Prague. In my research, I employ various methods of plant genomics and molecular biology, including plant phenotyping, confocal and light microscopy, and molecular cloning. I am currently engaged in two research projects focusing on Arabidopsis thaliana as a model organism. One of my projects examines the impact of tRNA modification t6A on plant development. In my other project, I explore the relationship between mRNA modification m6A and auxin response.

N6-methyl adenosine (m^{6}A) is the most common mRNA modification that plays a critical role in regulating transcript stability and overall gene expression. Our study investigates the connection between m^{6}A and auxin pathways in *Arabidopsis thaliana*. We characterize the auxin-related phenotypes and auxin resistance of the hypomorphic mutants with the

impaired status of m^6A. Next, through an extensive analysis of the published transcriptome-wide m^6A datasets, we demonstrate that m^6A is present in many auxin-related transcripts. Quite exceptional is the group of SAUR transcripts that, in contrast to other categories, lack methylation marks completely. We further show that transcripts encoding the key components of the auxin signaling pathway, including the TIR1/AFB receptors and ARF transcriptional regulators, might be particularly affected by the m^6A-mediated regulation. We also observe the correlation between the methylation status of IAA amidohydrolase transcripts and the moderately altered internal levels of IAA metabolites. In addition, we reveal that the lack of m^6A leads to defects in the endodermal patterning of the primary root due to the impaired timing of periclinal cell divisions. Notably, these defects can be reverted by inhibition of auxin signaling. Overall, our data highlight the crucial role of m^6A in regulating multiple aspects of auxin-dependent processes.

The Role of Long Non-coding RNAs in BCR-mediated CLL Activation

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My name is Pedro Zeni and I am a Biomedical Scientist. As a PhD candidate, my research focuses on long non-coding RNAs in B cell malignancies. I'm passionate about science and I believe it to be the best tool for decision-making and describing the world around us. Throughout my career, I hope to contribute to our understanding of cancer biology and ultimately improve patient outcomes.

B cell Receptor (BCR) plays a pivotal role in providing maturation and survival signals for B cells. However, dysregulation of the BCR pathway is a fundamental characteristic observed in numerous B cell malignancies, including chronic lymphocytic leukemia (CLL), revealing its importance in disease progression.

Despite the absence of recurrent mutations found in the BCR-related genes of untreated cases, BCR inhibitors have shown a universal clinical response in CLL patients. We and others have shown that short non-coding RNAs, namely microRNAs, can (dys)regulate the BCR signaling propensity, but it is still unclear if long non-coding RNAs (lncRNAs) play a role in BCR

activation. Hence, we hypothesized that lncRNAs could be involved in BCR-mediated CLL activation.

To address our hypothesis, we performed differential lncRNA expression analysis in CLL cells from patients treated with BCR inhibitors and cross-validated in CLL intraclonal subpopulations with high BCR activity ($CXCR4^{dim}$ $CD5^{bright}$) vs. low BCR activity ($CXCR4^{bright}$ $CD5^{dim}$). We found 12 lncRNAs related to the BCR pathway inhibition/activity. Out of these lncRNAs, we selected a lncRNA that belongs to a class of lncRNA called long intergenic non-coding RNA (lincRNAs) which often play a role in trans-activating signaling pathways.

The studied lncRNA was upregulated upon BCR activation (BCR crosslinking with anti-IgM) in CLL cells and had impaired upregulation when cells were treated *in vitro* with BCR inhibitors (ibrutinib/idelalisib). In line with this data, the lncRNA was also downregulated in CLL patients undergoing BCR inhibitor therapy (p=0.003, fold-change=3.84). Also, CLL patients with higher expression of the lncRNA have longer survival than those with relatively low levels (n=100, p=0.04, HR = 2.28; median survival of 9.7 years vs. 16.8 years).

To understand these observations, we transcriptionally repressed the lncRNA using dCAS9-KRAB system in MEC1 cell line. Surprisingly, the engineered cells showed higher BCR responsiveness, as evidenced by the increased calcium flux (FLUO-4 assay) followed by BCR activation. RNA profiling of the lncRNA-depleted cells showed a decreased expression of genes regulating BCR signaling and increased expression of genes involved in mitochondria respiration metabolism. Preliminary data suggest that although the cells transcriptionally enhance metabolic gene expression, the lncRNA-depleted cells have a higher glucose dependence and lower mitochondria respiration fitness. Altogether, these results indicate a possible mitochondria respiration deficiency, which the cells try to compensate for transcriptionally.

We further validated the interaction with EZH2, a known epigenetic regulator. At the moment, we are currently investigating how this interaction impacts Polycomb repressive complex activity by mapping the H3K27me3 markers (Cut&Tag) on the engineered cells and acquiring a comprehensive overview of genome accessibility using ATACseq. Additionally, we are optimizing an unbiased approach (RNA Pull Down assay) to identify molecular interactors that can possibly link the phenotypes. Consequently, we expect to be able to describe the lncRNA function in CLL cells.

In summary, the studied lncRNA is directly regulated by the BCR activation and potentially acts in a negative feedback loop to limit BCR-mediated CLL activation.

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Collective Variables based enhanced MD promotes the sampling of non-canonical DNA dynamics

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Zhengyue Zhang is a 4th-year PhD candidate at Masaryk University, CEITEC, studying structures and dynamics of Nucleic Acids with Molecular Dynamics (MD) simulations. He has done three scientific projects on the dynamics of both DNA and RNA non-canonical structures, resulting into two publications and one ongoing manuscript. Zhengyue was motivated by the advanced MD sampling methods giving high resolution insights which are not achievable for the experiments, aiming to understand the principal behind the Nucleic Acids dynamics during his PhD career. When he is free from work, Zhengyue enjoys wandering in nature and numerous museums in Europe, with 24 European countries visited so far.

The understanding of DNA structure is a cornerstone of investigations on biological processes, structure-based drug discovery, and DNA

nanomaterial designs. The diversity of DNA structure and dynamics, however, has brought major obstacles towards further studies and applications. Despite various technical/experimental developments in Structural Biology, reaching high time- and scale-resolution details of DNA structures remains challenging. Therefore, with the potent computational capability, Molecular Dynamics (MD) is a powerful tool to fill the information gap.[1]

By focusing on two types of non-canonical DNA structures, Holliday Junction (HJ) and Guanine-Quadruplex (G4s), which represent the complicated DNA structural dynamics, as examples, and combining MD simulations with Collective Variables (CVs) based enhanced sampling methods, we observed abundant conformation changes of HJ and G4s at atomistic level and suggested potential intermediates on profiles of DNA structural transitions. During the explorations, we also raised attention to the inaccurate Lennard-Jones parameters and suggested system-specific modifications on the OL15 DNA force field.[2]

Overall, our studies demonstrated the power and perspective of MD simulations and CVs-based enhanced sampling methods, which could inspire and cooperate with the experiments for future breakthroughs in the studies of DNA structure and dynamics.

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