ABSTRACT BOOK

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Disclosing GH3-mediated inactivation mechanisms that govern auxin homeostasis in plants

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The GH3 family of amido synthetases catalyze ATP-dependent conjugation of acidic phytohormones with amino acids, producing either active or inactive hormone forms. In the context of the auxin inactivation framework, GH3s mediate the first step by forming IAA-aa. Next, IAA-aa can undergo hydrolysis back to IAA *via* IAA-aa hydrolases (ILR/ILL) or oxidation to oxIAA-aa (DAO). These biochemical processes occur in distinct subcellular compartments. IAA conjugation and oxidation occur in the cytosol, while IAA-aa hydrolysis occurs within the endoplasmic reticulum (ER). The transport of IAA-aa from the cytosol to the ER has not yet been demonstrated, however, our *in silico* predictions reveal significant interactions between IAA-aa and ER-localized IAA transporters, PIN5 and PIN8, indicating potential transport into/out of the ER. Recent evidence suggests that the nucleus may also be involved in auxin homeostasis. DAO was reported to have nuclear and cytoplasmic localization and specific IAA metabolites were detected in sorted nuclei. We have generated GH3-GFP stable Arabidopsis transgenic lines and observed GH3 presence not only in cytosol, as previously documented, but also within the nucleus supporting its direct role in auxin metabolism.

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An unusual reversible prenylation on human ALDH9A1

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Protein lipidation is a post-translational modification controlling their cellular localization, trafficking, and function. In particular, protein prenylation is a C-terminal modification on proteins bearing canonical motifs catalyzed by prenyltransferases. Prenylated proteins have been of interest due to their associations with various diseases. Here, we describe the discovery of prenylation of a prenylated protein, ALDH9A1, which lacks any apparent prenylation motif. This enzyme was initially identified through chemical proteomic profiling of prenylomes in various cell lines. Metabolic labeling with an isoprenoid probe using overexpressed ALDH9A1 revealed that this enzyme can be prenylated inside cells but does not respond to inhibition by prenyltransferase inhibitors. Site-directed mutagenesis of the key residues involved in ALDH9A1 activity indicates that the catalytic C288 bears the isoprenoid modification likely through an NAD+-dependent mechanism. Furthermore, the isoprenoid modification is also susceptible to hydrolysis, indicating a reversible modification. This modification originates from endogenous farnesal or geranygeranial, the degradation products of prenylated proteins and results in a thioester form that accumulates. This novel reversible prenoyl modification on ALDH9A1 expands the current paradigm of protein prenylation that may also serve as a novel mechanism for controlling enzyme function.

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Crosstalk between light and ABA signaling pathways in plant responses to abiotic stresses

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In recent years, research focused on plant stress is becoming more and more popular due to climate change on Earth. Environmental stresses such as drought, extreme temperatures and high salinity are the major factors reducing productivity of crop plants. Negative effects of abiotic stress on plants lead to changes in its metabolism, to reduction of the growth or finally to death of the plant. Scientists suppose that exposure to abiotic stress factors can reduce crop production by up to 70%. In the future, it will be necessary to breed tolerant plants able to adapt to new external conditions. Therefore, understanding of mechanisms involved in plant responses to environmental stresses is very important to solve the agronomic problems.
Plant hormones, especially abscisic acid (ABA), play a critical role in adapting plants to environmental conditions. When exposed to abiotic stress, plants accumulate ABA. At the same time, the level of ABA in plant is altered by light conditions, especially by blue light. This fact raises the question what role does blue light play in plant responses to abiotic stress. In our project, we investigate molecular mechanisms by which blue light crosstalks with ABA during responses to abiotic stress factors. We apply genetic approach consisting in analysis of tomato mutants (*Solanum lycopersicum*) with defects in various stages of ABA biosynthesis and/or in genes coding for blue light photoreceptors. To find out the role of light and ABA in plant responses to abiotic stress, we study growth responses of the mutants to various effectors (salt, mannitol, exogenous ABA) and under various light conditions.
It is hypothesized that salt stress leads to decreased seed germination concomitantly with higher ABA production by seeds. But the results are contradictory. In ABA-deficient mutant (*flacca, sitiens*) and mutant *hp1 (high pigment 1*) the responses to salt stress were not the same. The tomato mutant *hp1* shows hypersensitive responses to blue light and red light and according to our results the mutation lead to reduction of ABA in seeds. Similarly like *sitiens*, *hp1* was less sensitive to NaCl than corresponding cultivars in the dark, in blue light or red light. On the contrary, mutant *flacca* seems to be more sensitive to NaCl than corresponding cv. Rheilands Ruhm, in all light conditions. Furthermore, we discuss the possibility that ABA can interact with other hormones, such as ethylene, gibberellin in plant responses to salt stress.

Using mass spectrometry for study of salicylic acid metabolism in plants

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Plant immunity is defined as the capacity of plants to prevent or ward off biological attacks by pathogens. It involves the recognition of the pathogen by specific receptors and the triggering of signalling pathways leading to processes that help plants to defend themselves. The defence signalling is mediated by cross-communication of groups of plant hormones. Salicylic acid (SA) is one of the most pronounced plant hormones involved in control of immunity and defence mechanisms in plants. Defence related SA accumulation comes from its biosynthesis, transport and possible release from some of its metabolites. The involvement of SA biosynthetic pathways as well as extend of SA metabolism in various plant species are still under investigation. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is one of the most commonly used analytical technics in plant hormone quantification. The selectivity and high sensitivity enables to track typically low concentrations of

phytohormones. The comprehensive multiclass phytohormone LC-MS/MS profiling methods usually include only SA (as the active compound) from the group of SAs. LC-MS/MS methods for determination of defence related phytohormones focus on SA, or some on its biosynthetic precursors or metabolites. In this study, we focus on identification of new SA metabolites and development of liquid chromatography mass spectrometry methods.

The Yang cycle as a link connecting ethylene and polyamine biosynthesis

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The Yang cycle comprises a set of reactions that maintain a strictly regulated balance of sulfur-containing compounds in plant cells and organs. Its first intermediate, methionine, is an essential building block of protein synthesis; however, the majority of the amino acid's cellular content is activated by ATP to form S-adenosylmethionine (SAM). SAM serves as a methyl donor in enzymatic reactions in both plants and mammals, and its aminobutyrate moiety is utilized in many biosynthetic reactions. Conversion of SAM into 1-aminocyclopropane-1-carboxylic acid (ACC) is the first step of ethylene synthesis, followed by ACC oxidation and immediate ethylene production. In another pathway, decarboxylated form of SAM is introduced to facilitate the production of certain polyamines.
Both ethylene and polyamines play a central role in growth and developmental processes, as well as regulate the plant's response in tolerance of biotic and abiotic stresses. In order to study the complex relationship of these growth regulators in relation to various aspects and conditions, it is therefore beneficial to analyse these groups of compounds comprehensively in terms of their concentration. However, verified profiling methods that would provide a connecting link between these pathways are lacking.
Despite the compounds' diverse chemical properties and various endogenous abundances, both of which present a challenge for effective extraction and quantification, our proposed method enables us to detect most of the known analytes of the cycle. A simple approach for sample extraction and UHPLC-MS/MS detection allows for a straightforward quantification of intermediates of the Yang cycle and related compounds, with possible modifications for subsequent polyamine and phytohormone analysis.

Effect of stress on ethylene and other phytohormone levels in Arabidopsis

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Ethylene, a vital gaseous phytohormone, orchestrates a myriad of plant developmental processes, prominently including responses to environmental stresses. This phytohormone not only regulates growth and senescence but also plays a crucial role in mediating plant responses to abiotic stresses such as drought, salinity, and temperature extremes.
By modulating gene expression and signaling pathways, ethylene enhances plant resilience and adaptability, thereby influencing overall crop productivity. A comprehensive understanding of ethylene's mechanisms in stress response is imperative for advancing agricultural practices aimed at mitigating the impacts of climate change on crop yield and stability. Exploring these mechanisms offers promising avenues for developing stress-tolerant plant varieties, which is essential for ensuring food security in an increasingly volatile environment (Lin et al., 2009; Zhang et al., 2022).
We aim to how altered ethylene production in Arabidopsis mutants affects the profiles of various phytohormones, especially under abiotic stress conditions. our research employs a validated derivatization

method to precisely measure levels of ethylene's precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), alongside other phytohormones. Our findings reveal significant interactions between ethylene, ACC, and other phytohormones, highlighting how changes in ethylene production can influence overall hormonal balance and stress response mechanisms in plants. This research underscores the potential of advanced analytical methods for comprehensive phytohormone profiling, providing insights that could lead to the development of crops with enhanced stress tolerance and optimized growth.

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Effects of new cytokinin derivatives and plant biostimulants on seed germination, growth and development of food crops.

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The current global population's demand for food necessitates continual growth in food production. Conventional agricultural practices rely heavily on the widespread application of chemical fertilizers and pesticides, posing risks such as soil depletion and environmental degradation. Plant biostimulants encompass substances or microorganisms that, irrespective of their nutrient content, can be applied to plants to enhance seed germination and foster developmental growth. Concurrently, they improve nutritional efficiency and have the potential to mitigate the adverse effects of conventional farming. These substances may offer an affordable solution, suitable for local preparation and application by farmers across various regions.
Smoke-water, derived from enriching water with smoke from burned plant material, contains karrikinolides, growth regulators that induce dormancy release and enhance seed germination and post-germination growth in numerous plant species. Vermicompost, resulting from the decomposition process of organic waste by earthworms, yields leachate rich in growth regulators and nutrients, enhancing stress resistance in plants. Seaweed extracts, commercially marketed as Kelpak® from species such as Ecklonia maxima or Macrocystis pyrifera, also contain growth regulators and nutrients, thereby enhancing seed germination, nutrient uptake, and overall growth and development.
While these potent biostimulants have demonstrated significant effects individually, their combined impact remains largely unexplored. Therefore, our study aims to investigate the synergistic effects of these substances on seed germination, growth, and development in cereals and horticultural crops like carrots, peas, cucumbers, or cabbages. We will explore various modes of application to assess their effectiveness comprehensively, providing insights into sustainable agricultural practices and optimizing crop yield across diverse farming systems.

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Towards the development of a novel generation of plant growth retardants

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In modern agriculture, the utilization of fertilizers, pesticides, and herbicides plays a crucial role in shaping the growth and development of field crops. However, their usage comes with environmental repercussions, prompting the quest for alternative, eco-friendly options. Given the significant role of gibberellins (GAs) in plant growth regulation, they are being considered substitutes for synthetic counterparts.[1,2] In principle, the control of plant growth can be influenced in two ways, by the addition of exogenous addition biologically active GAs (increase of endogenous GAs – increase of growth, germination) or by the application of growth retardants (reduction of endogenous GAs – shutting down the plant growth).[3,4]
Recently we devised and tested a new gibberellin-based plant growth inhibitor, which acts as a competitive antagonist to bioactive GAs. After intensive field trials optimization (dosage, type of application, and determination of the optimal vegetative period of application; three years of field trials) an optimal protocol for barley (20% increase (t/h) in grain yield) and wheat (7% increase (t/h) in grain yield) was developed.
Currently, our focus lies on enhancing the bioavailability and water solubility of our leading compound. To tackle the former, we've synthesized a fluorinated derivative aimed at improving the molecule's migration through cell membranes to the active site. Initial enzyme assays suggest superior inhibitory activity compared to existing compounds. The second objective aims to prepare a more soluble derivative of anti-gibberellin in the form of its ammonium and potassium salts. Both higher solubility and bioavailability should lead to a reduced dose when applied to plants.
In this contribution, we present the latest results obtained during this project.

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Are phenylacetic acid and indole-3-acetic acid metabolic pathways mirror images?

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The growth and development of plants are regulated by a group of bioactive compounds known as plant hormones (phytohormones). These substances function optimally within a narrow concentration range, necessitating strict regulation of their levels in plant cells and organs. This regulation primarily occurs through biosynthesis, metabolism and transport mechanisms. Among the diverse groups of phytohormones, auxins were the first to be identified due to their profound effects on plant tropisms. Two essential endogenous auxins are indole-3-acetic acid (IAA) and phenylacetic acid (PAA). These compounds exhibit biological activity only in their free, unconjugated form, as enzymatic reactions produce metabolites that serve as temporary storage and transport forms, as well as degradation products. This conversion occurs either through irreversible oxidation or reversible conjugation with amino acids and sugars. Thus far, the metabolism of PAA and IAA appears to be quite

similar, as the same enzymes catalyse the synthesis of identical conjugates.
In recent years, many new discoveries have been made in the field of auxin metabolism, expanding our knowledge about conjugates and catalysing enzymes. However, most of this research is connected to IAA and Arabidopsis thaliana as a model plant. Consequently, the objective is to expand the comprehension of the metabolic processes of PAA across a range of land plant species and their organs. By employing liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), novel PAA endogenous metabolites were identified. Subsequent experiments involved complex IAA and PAA metabolite profiling in various land plant species and their organs, as well as the elucidation of enzymes responsible for these reactions through the utilisation of bacterial enzymatic assays.

Equilibrative Nucleoside Transporter 1 drives cytokinin riboside uptake in Arabidopsis roots

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The transport of cytokinins plays a crucial role in understanding plant growth, development, and responses to environmental stresses. In recent years, several cytokinin transporters have been characterized, highlighting their involvement in processes such as long-distance cytokinin transport (Zhang et al., 2014; Ko et al., 2014; Zhao et al., 2023) or lateral root development (Tessi et al., 2021; Tessi et al., 2023). Here, we introduce Equilibrative Nucleoside Transporter 1 (ENT1), which not only actively participates in cytokinin transport but also stands as the primary candidate for cytokinin uptake by roots. Among all members of the ENT family, ENT1 exhibits the highest expression levels (Cornelius et al., 2012); our GUS-promoter analysis also confirmed high expression in the epidermis of the root apical meristem.
Our findings using the transport assay in the BY-2 inducible ENT1 line revealed that ENT1 facilitates the transport of cytokinin ribosides across the plasma membrane. Furthermore, by employing isotopically labeled *t*ZR, we confirmed transport across the tonoplast membrane. Through the generation of a functional line expressing ENT1 under its native promoter/terminator, we incorporated GFP between selected amino acid residues downstream of the predicted acidic di-leucine motif to ensure proper recognition of this critical signaling sequence. The expression of *pENT1::ENT1-T53-eGFP-K54-3'-UTR* exhibited dual localization on both plasma membrane and tonoplast.
Upon expressing the *pENT1::ENT1-T53-eGFP-K54-3'-UTR* construct in the Col-0 background, this line exhibited higher and specific sensitivity towards cytokinin ribosides, but not free cytokinin bases. Notably, even more pronounced sensitivity to cytokinin ribosides was demonstrated utilizing the inducible *XVE::ENT1* lines in *TCSv2::NLS-3XVenus* background. Additionally, root-feeding experiments revealed enhanced uptake of isotopically labeled cytokinin ribosides into the root, as documented by autoradiography using 3H-*t*ZR.
The robust and specific expression pattern of *ENT1* in the root epidermis suggests its role as a gateway for cytokinins from the surrounding environment. Furthermore, its expression in protoxylem cells raises the possibility of involvement in long-distance cytokinin transport, with specificity for both iPR and *t*ZR.

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Bioactive lipids: synthesis and application of nitro fatty acids

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Nitro fatty acids (NFA) are a class of highly potent compounds that are formed endogenously and exhibit various bioactivities. They act as lipid mediators with particular importance in inflammatory processes. They have been structurally characterized and quantified in plasma, red blood cells, and urine from healthy and hypercholesterolaemic individuals.[1] Nitroalkene functionality is a less explored warhead in drug candidates since it has been approved by FDA only in 2016. NFAs are formed by the reaction of unsaturated fatty acids with reactive nitroxide-derived species.[2] NFAs can modulate several different signalling pathways that are important in both initiating and terminating inflammation. Their potent anti-inflammatory and cell-protective effects have already been demonstrated in several animal studies.[3–5]

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Similarities and differences in the action of aromatic (N6-benzyladenine) and urea (MTU) antisenescence compound in Arabidopsis (from genes to physiological response)

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Senescence, a natural process in plants, can be delayed through the application of cytokinins and their derivatives. In our study, we compared the effects of two substances: BAP (6-Benzylaminopurine), used as a control, and MTU (1-(2-methoxy-ethyl)-3-1,2,3-thiadiazol-5-yl urea). Through a Gene Ontology (GO) enrichment analysis on separate Arabidopsis leaves, MTU showed heightened responses to light stimulus and enhanced photosynthetic activity.
Using these findings, we investigated how both substances influenced senescence in darkened leaves over varying time periods (2, 5, 7, 9, and 12 days). We closely monitored parameters such as photosystem II efficiency, chlorophyll levels, photochemical/non-photochemical quenching, and cyclic electron transport.
Additionally, we examined the effects of MTU and BAP on Arabidopsis 7-day-old seedlings. Our focus was on evaluating their influence on root growth and development. Furthermore, we employed qPCR analysis to determine their activation of ARR genes.
Our study sheds light on how cytokinin (CK) signaling modulates the transcriptome, particularly in relation to photosynthesis and light response reprogramming in darkened leaves. This understanding offers valuable insights into CK-mediated anti-senescence mechanisms in plants, contributing to the identification of new traits for developing more resilient crops amidst the challenges posed by climate change.

Water-Soluble Cytokinins: Exploring Their Crystal Structure and Impact on Crop Yield

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Cytokinins (CK) are natural compounds with various effects on plants, including delaying of leaf senescence, promoting cell division, stimulating cell growth, and many more [1]. A notable characteristic is that aromatic CKs are generally insoluble in common solvents, significantly limiting their agricultural application [2]. My research aimed to enhance the water solubility of CKs while also investigating their crystal structure. Water solubility of CKs increased dramatically by their reaction with methanesulphonic acid. The newly synthesized mesylates also exhibited enhanced efficacy on barley and wheat in both in vitro and in vivo experiments in comparison with natural CKs. They were found to protect PS II by reducing chlorophyll degradation in barley leaf segments undergoing artificial senescence [3]. Most importantly, these compounds significantly increased grain yield of barley and wheat (up to 15%), and boosted the number of strong, productive tillers by up to 62% compared to untreated control in field experiments. At last, ^{15}N isotopically labelled CKs and their mesylate salts were synthesized for future experiments to determine bioavailability of those compounds.

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Correland^{SW}: Correlation networks from LC-MS data

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The significance of software development for processing of high-density MS data has become essential in recent years. Metabolomic analysis is a typical example of analytical technique that provides a large amount of information related to chemical entities present in biological samples. Software tools that support network creation based on the large number of variables, such as Cytoscape / MetScape and JASP, are available for biological research. However, none of these allows one step construction of correlation networks. Correland is a software tool designed in MATLAB for the analysis of metabolomic data. Its purpose is to facilitate the visualization of the relationships among ions. It works based on the Pearson correlation coefficient, which is rescaled for better visualization. Users have the option to customize the graphical output by setting a number of parameters, such as area threshold, p-value, and ion-grouping capability. Graphical output can be saved as an image (.svg, .png, .pdf, .tif) or, alternatively, the information about the end nodes, weights and correlation coefficients can be exported as an xls file. Furthermore, the software allows the generation of a correlation network for any input dataset for which Pearson correlation coefficient can be calculated. Due to the sophisticated algorhitms, Correland can be readily utilized by an inexperience user.

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Technologically efficient approaches to the preparation of the cytokinin conjugates with the anti-senescence effects and potential application in human skin models

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The aim of this presentation is the synthesis and determination of biological activity of the new kinetin derivatives with potential antisenescence and anti-aging effect. Theoretical part includes general information about cytokinins, their classification and practical utilization. Separated sections characterize antisenescence and anti-aging activity of cytokinins. The following sections are focused on synthesis and the characterization of C2-substitued cytokinin derivatives and overall summary of the results of diploma thesis. In the experimental part biological and chemical methods are listed and specific methods of chemical synthesis for the preparation of cytokinin derivatives are approximated and characterized.

Hormonal regulation of responses to drought and osmotic stress in Arabidopsis roots and shoots

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The role of phytohormones is crucial for plants to deal with abiotic stress. Although plants have developed effective strategies to respond to drought stress, the whole mechanism is not fully investigated yet. Abscisic acid (ABA) plays an important role in controlling stomatal aperture, while gibberellin (GA) signalling is also involved in the response to drought by regulating shoot growth and integrating information from other phytohormones signalling pathways. Drought stress caused by water restriction results in a redistribution of growth from shoots to roots through a reduction in GA levels. Another important environmental aspect is light, which influences the response of plants to drought and other abiotic stresses.

We are using gene expression and hormone analysis in combination with gene reporters and hormone mutants to investigate the cross-talk of GA and ABA signalling and their interaction in the response to drought and osmotic stress in seedlings of Arabidopsis thaliana under different light conditions. We are examining the importance of root-shoot communication in these responses through micrografting. The effect of these stresses on GA signalling is also being investigated using biosensors that enable GA accumulation and activity to be monitored at cellular resolution.

Determination of neuroactive steroids by ultrahigh-performance liquid chromatography–tandem mass spektrometry

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Neuroactive steroids are a group of steroid molecules involved in the regulation of nervous system functions. The levels of these substances depend on the physiological state of the individual (sex, age, diurnal variations, etc.) and are also influenced by various pathological processes in the nervous system (neurological and psychiatric diseases or injuries), and new insights can be gained by monitoring these changes.The aim of this work was to develop an analytical method for the simultaneous determination of selected neuroactive steroids in different biological matrices (human plasma samples were used for pilot experiments). Using this high-throughput and sensitive method, we were able to determine nine neuroactive steroidal compounds (pregnenolone, progesterone, 5α-dihydroprogesterone, allopregnanolone, testosterone, 5α-dihydrotestosterone, androstenedione, dehydroepiandrosterone and epiandrosterone) in 150 µl of human plasma by ultra-performance liquid chromatography with tandem mass spectrometry. The precision and accuracy of the method for all analytes ranged from 83 to 118% and 0.9 to 14.1%, respectively[1]. The described method could contribute to a deeper understanding of the pathophysiology of various diseases. It may also be useful in the search for new biomarkers and diagnostic options or new therapeutic approaches.

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Synthesis and estrogenic activity of BODIPY-labeled estradiol conjugates

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Novel BODIPY–estradiol conjugates have been synthesized by selecting position C-3-O for labelling. The conjugation strategy was based on Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) or etherification. Estradiol derivatives used as azide partners bearing an ωazidoalkyl function through C4–C8-long linkers have been prepared. CuAAC reactions of estradiol azides with BODIPY alkyne furnished fluorescent 3-O-labeled conjugates bearing the triazole ring as a coupling moiety. Williamson etherifications of 3-O-(ω-bromoalkyl)-17βestradiol derivatives with BODIPY-OH resulted in labelled conjugates connected with an ether moiety. Interactions of the conjugates with estrogen receptor (ER) were investigated using molecular docking calculations in comparison with estradiol. The conjugates occupied both the classical and alternative binding sites on human ER□□□with slightly lower binding affinity to references estradiol and diethystilbestrol. All compounds have displayed reasonable estrogenic activity. They increased the proliferation of ER-positive breast cancer cell line MCF7 contrary to the ER-negative SKBR3 cell line. The most potent compound 13a induced the transcriptional activity of ER in a dose-dependent manner in the dual luciferase recombinant reporter model and increased progesterone receptor's expression, proving the retained estrogenic activity. The fluorescence of candidate

compound 13a co-localised with the ERα. The newly synthetized labelled compounds might serve as a good starting point for the further development of fluorescent probes for modern biological applications.

Novel methods for determination of plant hormones in a plant tissue

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Phytohormones, including cytokinins, auxins, and ABA, are crucial in plant growth, development, and stress responses. Determining their concentrations in plant tissues, often very low, requires sensitive techniques. Ultra-high performance chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) is the gold standard for such analyses. Recent advancements include ultra-high performance supercritical fluid chromatography coupled with tandem mass spectrometry (UHPSFC-MS/MS) for rapid, sensitive analysis. Here, we introduce DisperSpin SPE, a novel method for purifying plant hormones, and compare CK profiling using UHPSFC-MS/MS with conventional UHPLC-MS/MS. We developed DisperSpin SPE using mixed-mode Oasis MCX® 30µm bulk sorbent for plant extract purification. Optimization involved solvent composition, dispersive SPE mode, and key efficiency parameters. Validation used Arabidopsis seedlings and poplar leaves, with robustness tested across six plant tissues. For CK profiling, we used an Agilent 1260 Infinity II LC/SFC hybrid system coupled with Agilent 6495B Triple Quadrupole. Chromatographic conditions were optimized with hybrid silica modified with 2-picolylamine as the stationary phase, exploring parameters like column temperature, back pressure, mobile phase composition, and make-up solvent. The DisperSpin SPE method effectively purified fifty plant hormones. Compared to conventional SPE, it used 15-fold less sorbent with high accuracy and precision (median values below 10%). UHPSFC-MS/MS enabled a 9-min analysis, tripling the speed of UHPLC-MS/MS, with quantification limits of 0.03–0.19 fmol per injection. Validation showed high accuracy and precision (below 15%). Applied to Arabidopsis thaliana, UHPSFC-MS/MS provided comparable CK metabolite profiles with improved peak symmetry and reduced matrix effects. We developed DisperSpin SPE and a rapid, sensitive UHPSFC-MS/MS method for analyzing 51 plant hormones, including CKs, auxins, and ABA. The UHPSFC-MS/MS method offers significant analytical speed advantages and reduced matrix effects compared to UHPLC-MS/MS, broadening the scope for fast target plant hormonomics.

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Modified betulinic acid has antiproliferative activity *in vitro*

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Based on the lupane scaffold, methyl betulonate and methyl 20,29-dihydrobetulonate were conjugated with

Reformatsky reagents to provide homolupanes extended at the C3-carbon atom to provide new bioactive derivatives. Further transformations of the functional groups afforded a series of derivatives with 2-hydroxyethyl and allyl alcohol moieties. Their varying antiproliferative activity in vitro was then investigated in four cancer cell lines and in normal human BJ fibroblasts. In cervical carcinoma HeLa cells, derivatives 5, 6 and 17 were the most promising with lower micromolar IC50s and no toxicity to fibroblasts, thus showing a high therapeutic index. In addition, induction of apoptosis was found in HeLa cells after 24 h treatment with compounds 5, 6, 13 and 29. This newly synthesized series is more interesting than the published lupane and homolupane triterpenes and saponins, due to their nontoxicity towards healthy human cells and stronger cytotoxicity to various cancer cell lines. This approach increases their potential as anticancer agents. In addition, using other bioassays we proved also more biological properties, such as antiangiogenic or anti-inflammatory activities.

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Induction of ethylene production by urea based cytokinin analogues

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This work will focus on testing and creating compounds, which exhibit cytokinin activity and are able to induce ethylene production, mainly on aromatic urea derivatives. Among the various goals of this work is also creating a better understanding of the mode of action of such compounds and creating compounds that will be able to be used in modern agriculture (mainly as cotton defoliants).
The methodology of compound testing will comprise of various biotests and methods of direct measurement of ethylene production by treated plants. The comparison of results of different methods would be then used to deduce relationships between molecular structures of tested compounds and their effects and modes of action. Currently developed biotests include induced ethylene production triple response biotest on pea plants, modiffied from one created by Jiroutová et al. (2019), biotest utilizing newly discovered fact, that application of active compounds may lead to formation of side shoots of pea plants. In the future, various other biotest including both pea and cotton will be created. In the final stage, the defoliation of adult cotton plants by active compounds will be tested. Various methods leading to quantiffying ethylene production will also be used. These methods will be based on utilizing GC-FID (possibly also GC-MS) and a novel method of photoaccoustic ethylene detection by ETD-300 detector.Using these method, ethylene production of germinating cotton and pea plants, arabidopsis and leaf cutouts form adult cotton plants under different conditions and treatments will be measured. Compounds, which are found active would be in silico modelled into binding sites of cytokinin receptors, COXs and proteins which may have some potential to bind cytokinins and induce reponses, but were previously unknown. The mechanisms of action of different compounds and posiible unknown regulatory pathways will then by inferred from the acquired data.

Identification and characterization of unknown sulfated metabolites from nature

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A search for unknown sulfated metabolites in plants, algae, and fungi was performed based on detecting neutral loss of SO_3 in UHPLC-QTOF-MS analysis. The search selected over 100 potential sulfated metabolites, followed by manual structure elucidation. Key fragments such as glycosides, phenols, and hormones were searched for in the MS/MS spectra, leading to the discovery of a group of mostly unknown sulfated phenolic acids in land plants for the first time (Supikova et al., 2022). The most important result was a match with a structure corresponding to auxin in *Urtica dioica*, suggesting a new IAA metabolic pathway involving sulfation. Based on the MS/MS spectrum, the structure was identified as 1-sulfoindole-3-acetic acid (SIAA) with sulfate attached via nitrogen. A similar N-sulfoindole structure was previously only known for glucobrassicin-1-sulfonate from woad (Elliott & Stowe, 1970). Since SIAA showed no auxin activity in the GUS assay, it may be involved in storage/degradation. However, its exact function is not clear. The SIAA concentration in *Urtica* was more than 100 times higher than that of IAA and was the highest of all analyzed IAA conjugates/precursors, except for Trp. The effects of different light conditions on IAA metabolites in Urtica were investigated. A significant increase in the SIAA/IAA concentration ratio was detected under continuous blue light (BL) compared to darkness, suggesting that SIAA is specifically affected by BL.

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Molecular mechanisms of interaction of light signaling pathways and aquaporins in plant tolerance to abiotic stresses

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Abiotic stresses are known to induce osmotic imbalance in plants and disturb the plant water homeostasis, leading to reduced growth and yield in agriculturally important plants. Aquaporins, the membrane intrinsic proteins facilitating and regulating the passive movement of water molecules down a water potential gradient, play a central role in maintaining a turgor and water transport in plants, and thus represent in plants a prerequisite for strategies against osmotic stress. Aquaporins can be divided into 5 major subfamilies according to their localization in plant cell. Plasma membrane intrinsic proteins (PIPs) with some of NOD26-like intrinsic proteins (NIPs) are located in plasma membrane and tonoplast intrinsic proteins (TIPs) are present in tonoplast of vacuoles. Aquaporins are also found in the endoplasmic reticulum as in the case of NIPs and small basic intrinsic proteins (SIPs). Light is sensed in plants by sophisticated unique photoreceptors. Perception of far-red and red light by plants is mediated by phytochromes. Plants use cryptochromes, phototropins and ZTL/ADAGIO family of photoreceptors to perceive blue and UV-A light. UV-B light is sensed in plants only by one photoreceptor called UVR8. These photoreceptors are involved in a wide range of developmental and physiological processes and are also influential in plant responses to abiotic stress. It is well known that gene expression of aquaporins can be regulated by abiotic stresses, but not much is known about how light signal pathways are involved in regulating water uptake via aquaporins, how light of specific wavelengths and intensities can influence aquaporin gene expression, and how light together with aquaporins can modulate plant responses to several abiotic stresses. Understanding these mechanisms would bring numerous benefits in the cultivation of agriculturally important plants and in breeding or biotechnological projects aimed at improving the productivity of these plants.

A-172 cell line treated with lipopolysaccharides as a model of neuroinflammation

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Appropriate models are needed to study neurodegenerative diseases, for understanding the molecular mechanism as well as testing of potential new drugs. In vitro models still provide a number of benefits and are particularly popular because they are easy to handle. They are often used in the testing of new compounds to find their potential protective activity. In recent years, the involvement of inflammation has come to the fore in the study of neurodegenerative diseases. Neuroinflammation is a response of the whole organism, and in the brain, it involves mainly astrocytes and microglia, which constantly communicate with each other. Mimicking these processes using single-cell in vitro culture seems to be a challenging task.
To create a simple single-cell model of neuroinflammation, we chose the human cell line A-172, which was derived from a human glioblastoma. Neuroinflammation can be induced by both extrinsic and intrinsic factors. External factors include lipopolysaccharides (LPS), a major component of the outer membrane of gram-negative bacteria, which was chosen to induce inflammatory responses.
In order to create the neuroinflammation model, a number of parameters had to be optimized, such as the number of cells per well or the appropriate LPS concentration. This was followed by the measurement of parameters that are key features of ongoing inflammation, such as production of reactive oxygen species and nitric oxide, cell death, cell proliferation and, last but not least, mitochondrial damage and their response. In particular, the study of mitochondria has yielded interesting results, which we decided to study further.
On the basis of the results obtained, we can conclude that the A-172 cell line after LPS treatment will probably not be a universal and versatile model for the study of neuroinflammation, however, some of its properties can be further developed and used. Testing of other parameters is also needed, for example, to determine the production of significant inflammatory markers such as IL-6, IL-1β and others.

Optimization of a method for use in studying mitochondrial complexes and supercomplexes in human cells

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Mitochondrial supercomplexes represent the higher level of organization of the respiratory complexes in the electron transport chain involved in cellular energy production. While many of the intricacies concerning these large protein assemblies are still to be unveiled, it is suggested that the organization of respiratory complexes into supercomplexes may increase the efficiency of electron transfer and reduce the production of reactive oxygen species. Understanding supercomplex assembly and disassembly is crucial for clarifying their role in general and their role in mitochondrial dysfunction, which has been reported in various neurodegenerative diseases, genetic diseases or cancer.
Not only is the structural and functional significance of supercomplexes still debated, so is the process of their assembly. Their dynamic nature makes them notoriously difficult to study. The heterogenity of mitochondria within different species, tissues and physiological states complicates the interpretation of supercomplex

function.
The study of supercomplexes has evolved significantly in recent years, utilising biochemical methods or high-resolution structural analyses using techniques such as cryo-electron microscopy or X-ray crystallography. This presentation focuses on the optimization of a method for studying mitochondrial complexes and supercomplexes in human cell lines ARPE-19 and BJ using electrophoretic methods. There exists a number of publications which focus on the so-called in-gel assays, which allow us to study the enzymatic activity of the complexes and supercomplexes after they have been separated on a native polyacrylamide gel. However, as of yet, there has been no optimized method developed, which makes comparisons of results from different publications difficult.

Alternative splicing of CRE1 receptor

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The plant hormone cytokinin plays a vital role in plant growth and development. While the molecular mechanism of cytokinin signal perception and transduction is well described, regulating this cascade in planta seems more ambiguous. The Histidine kinase receptor CRE1 (CYTOKININ RESPONSE 1)/AHK4 (ARABIDOPSIS HISTIDINE KINASE 4)/WOL (WOODEN LEG), is the crucial element of the cytokinin signaling cascade in Arabidopsis. The cytokinin-signaling pathway is under stringent negative feedback control executed at several levels. We discovered a novel CRE1 transcript variant with a negative feedback regulatory function. Cytokinin application induces intron retention within the CRE1 transcript, introducing a premature termination codon. The resulting transcript encodes for a truncated receptor lacking the receiver domain essential for activating the cascade. Our results demonstrate that the truncated receptor acts as a decoy competing for ligand binding with the canonical CRE1 receptors, ultimately attenuating cytokinin signaling. Thus, we propose a novel regulatory mechanism of cytokinin perception mediated by alternative splicing of CRE1 receptors.

The endoplasmic reticulum: Master regulator of auxin metabolism and transport

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Different cellular organelles play crucial roles in processes related to phytohormone action and intracellular homeostasis, such as signaling in the nucleus and hormone/metabolite storage in vacuoles. Recent findings suggest that the endoplasmic reticulum (ER) plays a central role in maintaining the subcellular homeostasis of the phytohormone auxin by regulating its distribution, metabolism, and likely its signaling. Auxin is a group of plant hormone. The most studied auxin is indol-3-acetic acid – IAA. IAA affects many developmental and growth aspect of plant including tropism, circadian rhythm and reaction to biotic and abiotic stress. Levels of free IAA are strictly regulated by biosynthesis, metabolism and transport. There are two types of active auxin transporters in Arabidopsis – extracellular and intracellular. Intracellular transporters include PIN5, PIN6, PIN8, PILS and WAT1. PIN5, PIN6, PIN8 and PILS are localized on endoplasmic reticulum (ER). PIN5 and PIN8 have antagonistic roles in IAA regulation.
To better understand the role of ER, we decided to isolate a highly enriched ER fraction from Arabidopsis thaliana ecotype Columbia preselected overexpressing mutants using our optimized protocol described in Včelařová et

al. (2021) and to determine the metabolic profile of IAA in the obtained fractions and whole plant samples. Unfortunately, the strength of gene expression in the overproducing lines was found to negatively affect the plant phenotype. For this reason, it was necessary to prepare new inducible lines.

Synthesis and characterization of silica nanoparticles functionalized with titanocene derivatives

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Titanocene dichloride is a well-known organometallic compound that has been tested in Phase II clinical trials in patients with metastatic breast carcinoma and advanced renal cell carcinoma. However, its activity was not very good due to the low solubility and stability of the compound in physiological medium. Therefore, the incorporation of this complex in nanoparticles could be an alternative to improve its pharmacokinetics. In this work, the design of systems based on mesoporous silica nanoparticles (MSNs) was carried out, as well as biological assays in order to compare the biological activity with the isolated complex and the influence of the chlorine atoms.
MSNs were synthesized by the sol-gel method when tetraethyl orthosilicate was used as silica precursor and hexadecyltrimethylammonium bromide was applied as surfactant. Titanocene derivatives were covalently incorporated on the surface of MSNs through grafting reactions trough mercapto ligand. Synthesized MSNs were characterized by analytical techniques such as FTIR spectroscopy (analysis of chemical bonds), TEM analysis (morphology of MSNs), BET analysis (textural properties), and ICP-MS analysis (content of functionalized metal). The biological effects of synthesized MSNs were tested on MDA-MB-231 and MDA-MB-468 triple-negative breast cancer cell lines by metabolic assay, CM-H2DCFDA assay, immunochemical analysis, and cell cycle analysis.
Treatment of MSNs functionalized with titanocene derivatives caused a decrease in the viability, mainly in MDA MB 468 cells. Further, the application of synthesized MSNs introduced ROS production with influenced cell cycle progression manifested by significant increase of the sub-G1 population after treatment with functionalized MSNs. Subsequent analysis of proteins involved in key signalling pathways revealed increased level of LC3B, the protein associated with the induction of autophagy.
MSNs were synthesized to determine how the appearance, or absence, of the chlorine atom influences the activity of titanocene derivatives and to compare the biological activity with respect to the isolated complex. The biological experiments showed that the chlorine atom is not necessary for titanocene dichloride activity, but its occurrence contributes to it. This information could be applied in future research focused on the development of novel and more efficient derivatives of titanocene dichloride supported on nanomaterials.

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Understanding drought response: ABA and PHOT signaling pathways in plant physiology

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While drought stress remains a global challenge, the comprehensive understanding of plant responses to this phenomenon remains incomplete. A pivotal aspect of water management in plants involves precise regulation of stomatal aperture, a nuanced process governed by multiple mechanisms. One such regulatory factor is the phytohormone abscisic acid (ABA), exhibiting increased levels in response to abiotic stresses like drought. Another crucial modulator of stomatal movement is blue light (BL), sensed through phototropins (PHOTs), leading to stomatal closure. This prompts an inquiry into the reciprocal influence between ABA and PHOT signaling pathways.
To explore the interplay between ABA and PHOT signaling pathways, we studied growth responses of PHOT-deficient mutants in Arabidopsis (phot1, phot2, and phot1/2 double mutant) via in vitro and in vivo experiments simulating drought stress. In vitro experiments monitored physiological parameters, such as root and hypocotyl growth, under different light conditions. Additionally, 5-week-old potted plants were exposed to drought and varying light conditions for 7 or 10 days. Subsequent analysis encompassed measurements of rosette size, rosette and root weight, phytohormone levels, gene expression related to drought stress and ABA metabolism, and proline content.