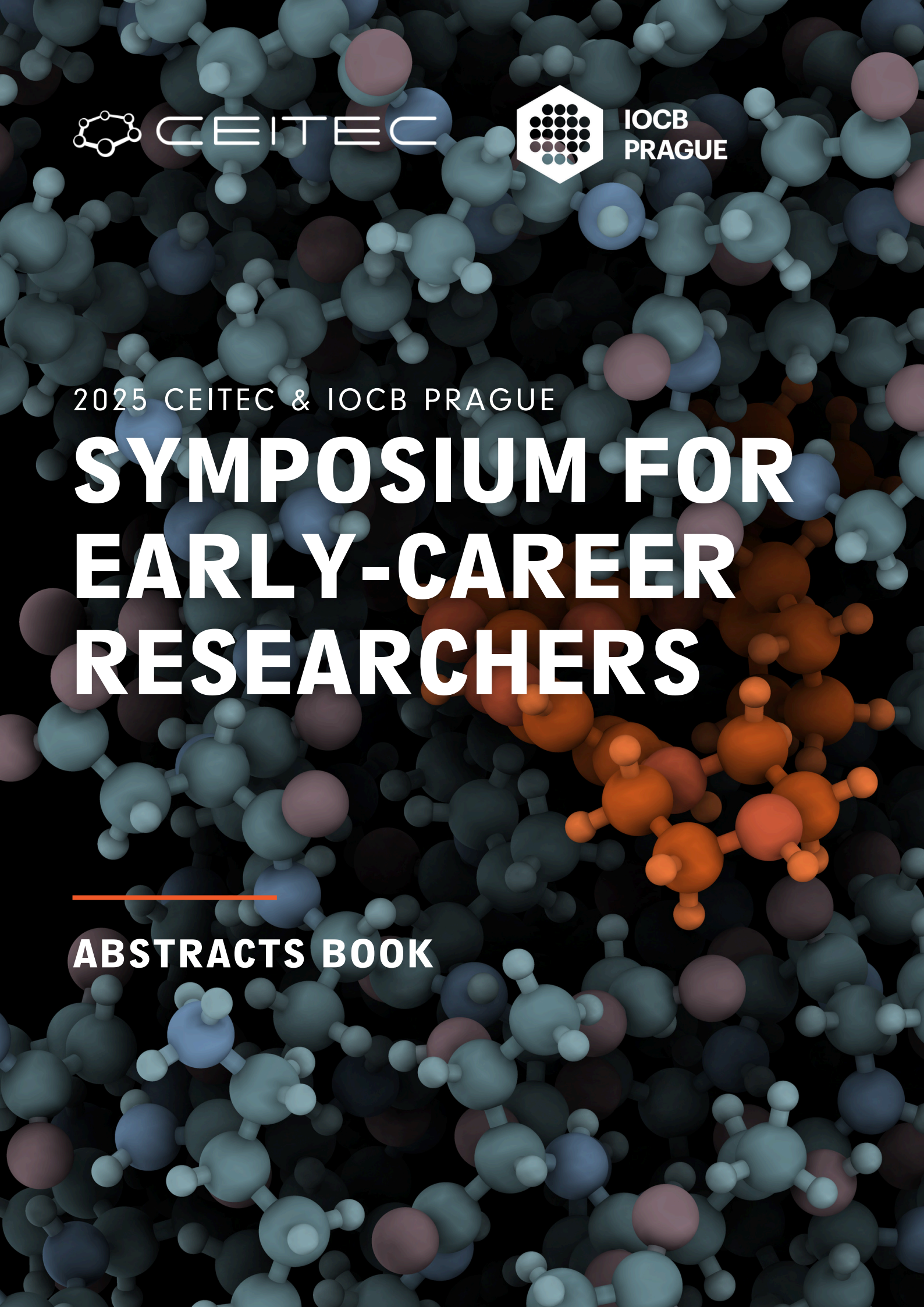

CEITEC
IOCB
PRAGUE
2025 CEITEC & IOCB PRAGUE
SYMPOSIUM FOR EARLY-CAREER RESEARCHERS
ABSTRACTS BOOK

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Note from the Organizers

We are delighted to welcome you to the CEITEC–IOCB Symposium for Early-Career Researchers, jointly organised by PhD candidates and postdoctoral fellows from CEITEC Masaryk University and IOCB Prague. This symposium aims to strengthen collaboration and exchange between emerging scientists from both institutions, fostering a vibrant and interdisciplinary research community. The programme features engaging talks by early-career researchers, two workshops on science communication and technology transfer, and invited lectures from Štěpánka Vaňáčová (CEITEC MUNI) and Hana Cahová (IOCB Prague). Participants will share their work through oral and poster presentations across a wide spectrum of disciplines, including life sciences, physics, and chemistry. The collegial atmosphere and inspiring setting will provide the ideal backdrop for scientific discussion, creativity, and new collaborations. We extend our sincere thanks to all participants, speakers, and organisers for their enthusiasm and contributions. We wish you an inspiring and enjoyable symposium.

The Organizing Committee

The Organizing Committee

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Special thanks to Michal Sýkora, Lucie Nepovímová, Eliška Janíčková, Karolína Krausová, and Blanka Collis

General Information

Accommodation and Meals

Attendees will be accommodated in shared double or triple rooms at the hotel, which also serves as the symposium venue. All meals, including breakfast, lunch, and dinner, as well as refreshments during coffee breaks, will be provided at the venue.

Poster Session

The poster session will take place on **Thursday evening from 5:30 p.m. to 7:30 p.m.** We are going to have an alternative "poster" session - what you need to prepare for it is a graphical abstract which grasps the core ideas of your research, maximum A3 size. Please bear in mind, the retreat attendees are from a wide span of fields and thus make your abstract and presentation at a level for an educated public.

Evening Networking & Entertainment

Following dinner on Thursday, attendees are invited to a relaxed social mixer. The evening will feature a science-themed pub quiz with small prizes for the winning team, providing a fun opportunity for networking and collaboration.

Workshops

The symposium includes two interactive workshops designed for your professional development: *How to Communicate with Impact* on Thursday and *Technology Transfer* on Friday.

Symposium Program

Day 1: Thursday, 16th October 2025

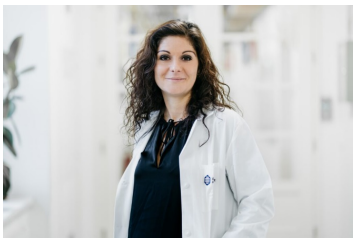
Time	Event
∼**07:45–08:00**	Departure (Prague/Brno)
∼**10:00**	Arrival
10:00 – 10:50	Registration
10:50 – 11:00	Welcome Word
11:00 – 11:45	Invited Lecture I: *Hana Cahová* (IOCB)
11:45 – 13:00	Lunch
13:00 – 13:15	Sponsor Talk I: *East Port*
13:15 – 15:15	Workshop I: *How to Communicate with Impact*
15:15 – 15:30	ECR Talk: *Eva Chocholová* (CEITEC)
15:30 – 15:45	ECR Talk: *Petr Janás* (CEITEC)
15:45 – 16:00	ECR Talk: *Jan Jurica* (IOCB)
16:00 – 16:30	Coffee Break
16:30 – 16:45	ECR Talk: *Mikuláš Klenor* (IOCB)
16:45 – 17:00	ECR Talk: *Alesia Melnikava* (CEITEC)
17:00 – 17:15	ECR Talk: *Dominik Musil* (IOCB)
17:30 – 19:30	Poster Session
19:30 – 23:00	Dinner & Networking

Day 2: Friday, 17th October 2025

Time	Event
07:30 – 09:00	Breakfast
09:00 – 09:45	Invited Lecture II: *Štěpánka Vaňáčová* (CEITEC)
09:45 – 10:00	Sponsor Talk II: *Alchimica*
10:00 – 10:15	ECR Talk: *Miguel Riopedre-Fernandez* (IOCB)
10:15 – 10:30	ECR Talk: *Jiří Rudolf* (CEITEC)
10:30 – 10:45	ECR Talk: *Lucia Tomovičová* (CEITEC)
10:45 – 11:00	ECR Talk: *Šárka Žaloudková Boháčová* (IOCB)
11:00 – 11:30	Coffee Break
11:30 – 13:30	Workshop II: *Technology Transfer* (CEITEC)
13:30 – 14:45	Lunch
15:00	Departure

Invited Speakers

Hana Cahová – IOCB Prague



Hana Cahová is head of a Senior Research Group at IOCB, the group research is focused on novel RNA modifications. Their research explores the functional roles of these RNA caps across various model systems, including bacteria and mammalian tissue cell cultures. Recently, they discovered a new class of non-canonical RNA caps by employing advanced LC-MS techniques, together with molecular biology approaches. Hana Cahová has received several awards, including the Otto Wichterle award and the Alfred Bader award for bioorganic chemistry. Since 2022, she is investigator of the prestigious ERC Starting Grant.

Štěpánka Vaňáčová – CEITEC MUNI



Prof. Štěpánka Vaňáčová, Ph.D., is a Senior Group Leader at CEITEC, Masaryk University, where she leads the RNA Quality Control group. Her research explores molecular mechanisms of post-transcriptional RNA modifications, focusing on noncanonical 3' tailing and adenosine methylation in RNA stability and degradation. A former EMBO Installation Grantee and elected EMBO Member since 2019, she has made significant contributions to understanding RNA regulation across eukaryotes.

Dinucleoside polyphosphates serve as RNA caps in bacteria and mammalian cells

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The 5´ termini of RNA are critical structures and remain among the least characterized RNA modifications. Until recently, only the canonical structures, NAD, and CoA were recognized as 5' RNA caps. We developed an LC-MS technique to detect a new class of 5' RNA caps—dinucleoside polyphosphates (Np_nN)—in isolated and digested bacterial RNA[1]. Our results show that bacterial RNA polymerases incorporate Np_nNs into RNA, and cryo-EM analysis revealed that both bases of Np_nN can pair with the DNA template during transcription initiation.
More recently, we focused on mammalian cells and identified two new types of Np_nN RNA caps: diadenosine tetraphosphate (Ap_4A)[2] and diadenosine diphosphate (Ap_2A). Our study demonstrated that RNA capped with Ap_4A is not translated and does not trigger an innate immune response. To further explore the role of Ap_4A-RNA, we are developing specific RNA sequencing methods. So far, we have established the Ap_2A-RNA Seq method, which allowed us to identify RNAs bearing Ap_2A at their 5' termini. Our findings indicate that, unlike other Np_nN-RNAs that are produced by RNA polymerases, Ap_2A-RNA is formed by a specific capping enzyme. Moreover, we observed that the intracellular concentration of Ap_2A-RNA is closely linked to oxidative stress responses.

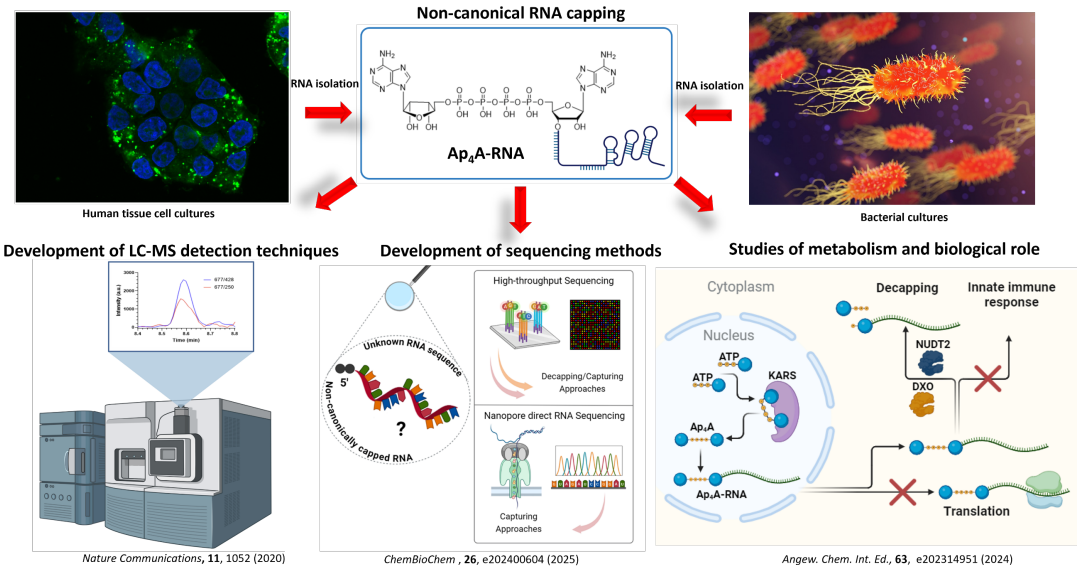


Figure 1. Exploration of Non-canonical RNA capping in various systems.

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Where RNA meets DNA: the role of m6A in DNA replication

Štěpánka Vaňáčová[a], Shwetha Krishna[a,b], Aleš Obrdlík[a], Anton Zuev[d], Praveenkumar Rengaraj[a,c], Maria Zlobina[a], Helena Peschelová[a,c], Veronika Kozlová[a], Michal Šmida[a], Jana Dobrovolná[d]

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Post-transcriptional modifications of coding and non-coding RNAs are a critical part of the regulation of gene expression in eukaryotic cells. The most prevalent internal mRNA modification is N6-methyladenosine (m6A) that regulates various steps of mRNA processing, stability, localization, and translation on the cellular level. In the whole organisms it is a key regulator during early development and gametogenesis from yeast to mammals and in disease, such as cancer or viral infections (1, 2, 3). Recently, m6A was also identified as a key regulatory factor of genome maintenance and stability. It is involved in DNA replication and repair by regulating gene expression and/or directly mediating repair processes (reviewed in 4).

M6A can be removed by the activity of the Fat mass and Obesity associated factors (FTO) and ALKBH5. We have previously observed that depletion of FTO, an RNA demethylase of m6A and m6Am, leads to increase of marks of damaged chromatin (5). This talk will present our recent findings that demonstrate a direct role of FTO in DNA replication. We show that FTO possesses genetic and physical interactions with DNA replication and repair factors. We demonstrate that FTO is physically present at sites of active replication and its demethylation activity is critical for normal replication fork progression. Under conditions of replication stress, FTO exercises a protective role over stalled replication forks to prevent the degradation of nascent DNA. Prolonged replication stress in the absence of FTO results in DNA strand breaks, possibly due to the collapse of stalled replication forks. Collectively, we uncover previously unknown role of FTO in the maintenance of replication fork integrity and provide a functional link between RNA modifications and DNA replication.

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Early-Career Researcher Talks

Selected Speakers

- Eva Chocholová – CEITEC MUNI
- Petr Janás – CEITEC MUNI
- Jan Jurica – IOCB Prague
- Mikuláš Klenor – IOCB Prague
- Alesia Melnikava – CEITEC MUNI
- Dominik Musil – IOCB Prague
- Miguel Riopedre-Fernandez – IOCB Prague
- Jiří Rudolf – CEITEC MUNI
- Lucia Tomovičová – CEITEC MUNI
- Šárka Žaloudková Boháčová – IOCB Prague

Palaeoproteomics of Dental Calculus: Exploring the Biomolecular Archive

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Dental calculus, a calcified form of dental plaque, entraps and preserves a wide range of particles that enter the oral cavity during life. As a rich source of information, dental calculus has become a focal point in biomolecular archaeology, greatly expanding our understanding of past populations. Biomolecular research has revealed insights into the ancient oral microbiome, respiratory and oral pathogens, diet, habitual practices, identity and migration, as well as medicinal use of plants.
In our project, we applied palaeoproteomic approaches to characterize the complex protein composition of ancient dental calculus. Samples originated from individuals buried at sites representing diverse geographical and temporal contexts. The most relevant and unique findings included plant-derived proteins with potential dietary or medicinal significance, together with numerous bacterial taxa, including putative pathogens.
These results emphasize the potential of palaeoproteomics to unlock biomolecular information from dental calculus, demonstrating its value as a unique archive for reconstructing health, diet, and cultural practices in past populations.

Engineering chromosomal rearrangements in *Cardamine hirsuta* using CRISPR/Cas: A new frontier in plant genome evolution

Petr Janas [a,b], Milan Pouch [a,c], Martin A. Lysak [a,b], Terezie Mandakova [a,b]

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Large-scale chromosomal rearrangements, such as deletions, inversions, and reciprocal translocations, are key drivers of plant genome evolution and speciation. The advent of CRISPR/Cas technology has revolutionized our ability to precisely engineer these rearrangements, enabling direct investigation of their genetic and phenotypic consequences. In this study, we employed CRISPR/Cas to induce targeted chromosomal rearrangements in *Cardamine hirsuta* (n = 8), a Brassicaceae model widely used in developmental, ecological, and evolutionary research. Using Agrobacterium-mediated transformation with SaCas9 and custom-designed gRNAs, we successfully generated multiple rearrangements, including a 3-kb deletion and inversion, as well as 0.1 to 2 Mb reciprocal translocation. These modifications were validated by PCR, Sanger sequencing, and chromosome painting, and were stably inherited in T2 and T3 generations. Our findings demonstrate the power of CRISPR/Cas for reshaping chromosomal architecture in *C. hirsuta* and other non-*Arabidopsis* genomes. Ongoing work focuses on position-effect variegation through engineered paracentric and pericentric inversions, and reciprocal translocations designed to restore the structure of the ancestral crucifer karyotype. These efforts will deepen our understanding of how chromosome rearrangements impact genome stability, evolutionary dynamics, and speciation. Beyond fundamental research, this approach opens avenues for applied plant genomics, including manipulation of genetic linkages and the unlocking recombination potential in crop breeding.

This project was funded by the Czech Science Foundation (grant numbers 24-11371S and 25-16796S to TM) and the ERDF Programme Johannes Amos Comenius (project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581).

COMBINING TURBO-GRIGNARD AND CRESOL-ASSISTED METHYLATION FOR SYNTHESIS OF GANAXOLONE ISOMERS: REGIO- AND STEREOSELECTIVE STRATEGY WITH COMPLEMENTARY NMR AND VCD PROFILING

J. Jurica,[a,b] H. Chodounska[a] , M. Budesinsky[a], M. Pazderkova[a], L. Bednarova[a], E. Kudova*[a]

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Neurosteroids are key modulators of brain function, with their pharmacological efficacy critically dependent on the precise regio- and stereochemistry of the hydroxyl group at position C3. To enhance metabolic stability and prolong bioactivity, alkyl substituent at C3 is introduced, as exemplified by the neuroactive steroid ganaxolone[1]. Achieving high regio- and stereoselectivity in such C3 alkylations remains a synthetic challenge.
Herein, we present synthetic strategies combining Turbo-Grignard and cresol-assisted Grignard methylation to enable regio- and stereoselective functionalization of 3-keto neurosteroid precursors. This approach affords control over axial and equatorial alkyl orientations with minimal side reactions, even for sterically hindered 5β-substrates, and significantly improves yields to near-quantitative levels compared to previous methods[2]. Detailed structural characterization of the synthesized ganaxolone isomers was accomplished through comprehensive 1D and 2D NMR spectroscopy, supplemented by vibrational circular dichroism (VCD) measurements. This complementary analytical profiling allowed unambiguous stereochemical assignment and confirmation of all 3α5β, 3α5α, 3β5β, 3β5α configurations. This regio- and stereoselective platform not only improves access to ganaxolone analogues but also broadens the chemical space for development of neuroactive steroid modulators, providing a versatile tool for designing next-generation ligands targeting $GABA_A$ receptors and related ion channels in the brain.

References

[1] Pinna, Graziano, Felipe B. Almeida, and John M. Davis. "Allopregnanolone in postpartum depression." Frontiers in global women's health 3 (2022): 823616.
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and Marshall Lee Morningstar.
SAGE THERAPEUTICS - WO2020/118060, 2020, A1 Location in patent: Paragraph 000625.

Mechanistic Insights into Cell-Penetrating Peptides Translocation Based on Charge Scaled Force Fields

Presenter Mikuláš Klenor[a], Collaborators V. Košťál [a], S. Fan [a], Hector Martinez-Seara[a], Principal Investigator P. Jungwirth*[a]

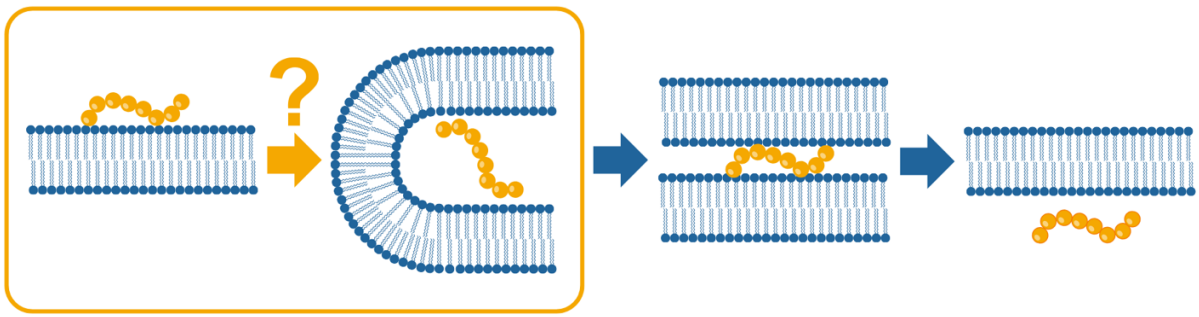
[a] *Pavel Jungwirth Group - Molecular Modelling, Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czechia*

Cell-Penetrating Peptides are short, highly positively charged peptides. Contrary to chemical intuition, they can passively traverse the hydrophobic core of cell membranes. To harness their potential as cargo carries, advanced simulations are required to understand the mechanism at atomic level.

We perform atomistic MD simulations of nonaarginine (R9) as a prototypical CPP. One of the early steps in R9 translocation involves inducing membrane curvature leading to membrane multilamerality[1]. To explore this process, we use model membrane mixture of DOPE, DOPS and DOPC. We investigate the interplay between the membrane curvature and R9 aggregation

To achieve reliable simulations, we develop force fields that enable an improved description of ions in MD simulations[2]. To target the well-known issue of full-charge force fields of overestimating interactions of divalent cations, we apply the theory grounded Electronic Continuum Correction (ECC), which incorporates electronic polarization by scaling of charges.

Our results demonstrate that ECC-modified CHARMM36 parameters for the POPC lipid, in combination with ECC-consistent ion and water models, effectively mitigate calcium overbinding to the membrane. The ECC-adjusted force field allows for a more accurate representation of ion–ion interactions without relying on empirical corrections or incurring the high computational cost associated with polarizable force fields.



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2. Nencini, R. et al. Effective Inclusion of Electronic Polarization Improves the Description of Electrostatic Interactions: The prosECCo75 Biomolecular Force Field. J. Chem. Theory Comput. 20, 7546–7559 (2024).

Cytokinin-Regulated DIRIGENT13 Enhances Root Architecture and Abiotic Stress Tolerance via Lignan Biosynthesis in *Arabidopsis*

A. Melnikava [a,b], Fr. Perreau [c], C. Paniagua [a,d], Gr. Mouille [c], D. Arnaud [a], J. Hejatko [a,b]

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DIRIGENT (DIR) proteins mediate stereoselective coupling of monolignols during lignan and lignin biosynthesis, yet their roles in development and stress adaptation remain poorly characterized. Here, we investigate *DIRIGENT13* (*DIR13*), a cytokinin-responsive gene in *Arabidopsis thaliana*, and its role in root growth, lignan biosynthesis, and abiotic stress resilience.

DIR13 is expressed in the root endodermis and margins of lateral root primordia. While not required for Casparian strip formation, *DIR13* promotes both primary and lateral root growth. Metabolomic profiling and imaging mass spectrometry showed that *DIR13* facilitates (neo)lignans biosynthesis.

Functional analysis of overexpression (OE) and mutant lines revealed that *DIR13* enhances salt and drought tolerance. Under salinity, *DIR13* OE maintained root growth, delayed leaf chlorosis, and exhibited higher photosynthetic efficiency. Chlorophyll fluorescence imaging (PlantScreen ™ SC Root System, Photon System Instruments) showed higher *Fv/Fm* values indicating better photosystem II (PSII) quantum efficiency in *DIR13* OE compared to wild type under stress. *DIR13* OE plants also accumulated more reactive oxygen species (ROS) under both control and salt stress, especially in the presence of cytokinin, linking *DIR13*-mediated lignan metabolism to ROS signalling and stress response. Under drought, *DIR13* OE plants had better survival, larger shoot area, and faster recovery upon rewatering. These phenotypic traits were supported by physiological data, including sustained *Fv/Fm* values during stress and recovery phases and indicating improved stress acclimation. Based on our findings we propose that DIR13 integrates cytokinin signaling, lignan metabolism, and ROS homeostasis to promote abiotic stress resilience.

Orthologs of *DIR13* are present in economically important *Brassica* species (*B. napus*, *B. juncea*, *B. oleracea*), widely cultivated for canola and mustard oil. These crops are rich in lignans such as pinoresinol and lariciresinol, compounds with antioxidant, anti-inflammatory, and cardioprotective properties. The presence of multiple *DIR13* homologs and high endogenous lignan levels suggest a conserved functional role in *Brassica*. Our results in *Arabidopsis* offer a molecular basis for enhancing stress resilience and nutritional value in oilseed crops, contributing to phenomics-driven strategies for climate-smart, health-promoting agriculture.

Supported by TANGENC (CZ.02.01.01/00/22_008/0004581) and LUAUS24277.

Selective Elimination of Myeloid CD64-Expressing Cells Using Synthetic Antibody-Drug Conjugates

Musil[a,b], Blažková[a], Brazdova[b], Krhutová[a], Starková[c], Zuna[c], Šubr[d], Kostka[d], Etrych[d], Šácha[a], Ormsby[a] and Konvalinka[a]

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Numerous therapeutic strategies, including antibody-drug conjugates, have emerged as a significant breakthrough in treating myeloid leukemic diseases. Among these, the high-affinity IgG FcγRI receptor CD64 has emerged as a particularly promising target due to its abundant expression on monocytes, acute myeloid leukemia cells, and tissue-resident MΦs, including tumor-associated macrophages (TAMs).

To explore a novel alternative to antibody-based immunotherapy, we developed anti-CD64 antibody mimetics (iBodies). These highly modular polymer-drug conjugates (PDCs) comprise synthetic HPMA copolymers decorated with a small cyclic CD64-peptide binder and a potent cytotoxic payload connected to the iBody backbone via cathepsin-cleavable linkers. This polymer-based backbone enables the attachment of various functional moieties and fine-tuning of the iBodies' pharmacokinetics and pharmacodynamics offering significant flexibility and biocompatibility for long lasting biodistribution while remaining non-immunogenic able to passively target tumor tissues through the enhanced permeability and retention effect.

These PDCs exhibited remarkable cytotoxic potency against CD64-expressing leukemia patient cells *ex vivo,* with no impact on circulating monocytes and immune cells lacking the CD64 expression. Importantly, in monocyte-derived macrophages, anti-CD64 cytotoxic iBodies by an apoptosis-dependent mechanism *in vitro* induced cell death of so called M2 macrophages, while leaving pro-inflammatory M1 macrophages untouched. Moreover, anti-CD64 iBodies do not exhibit off-target specificity or related cytotoxicity *in vitro* or *in vivo*.

Our findings suggest the potential of anti-CD64 cytotoxic iBodies as targeted myeloid cell therapies and opportunities for drug development and delivery in cancer immunotherapies.

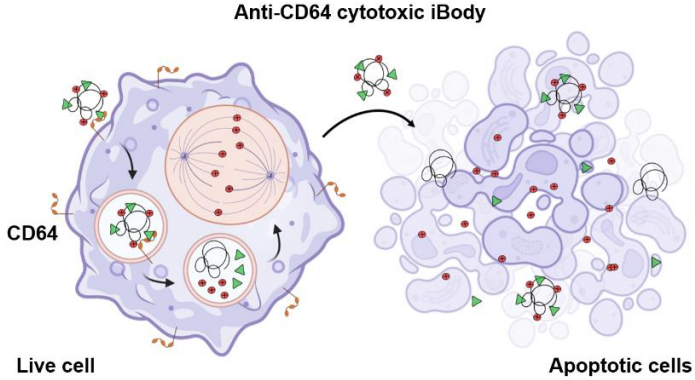


Figure 1. Schematic representation of mechanism of action of anti-CD64 cytotoxic iBodies.

Decoding the Glycocalyx with Molecular Simulations

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Molecular simulations have become a powerful tool in chemistry and biology. By providing unparalleled spatial and temporal resolution, they allow us to follow the motions of atoms and reconstruct complex chemical and biological processes.

In this talk, I will show how molecular simulations provide a unique perspective on one of the most challenging biological systems: the glycocalyx, a sugar-rich layer that coats most cells and acts as their first protective barrier. Understanding the molecular interactions within the glycocalyx is crucial for shedding light on processes such as viral infection, drug effectiveness, and cell–cell communication. However, the glycocalyx is highly flexible and disordered, making it difficult to study with traditional structural biology methods.

Here, molecular simulations can make a real difference. I will first introduce the basics of these methods, and then present examples where simulations have helped to unravel how glycans interact with proteins and peptides. Specifically, I will discuss an ongoing project in which we use computational tools to understand how glycans selectively bind to Siglec-15, a protein associated with cancer biology.

Unraveling the Role of Pseudouridylation in Plant Fertility

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Pseudouridine (Ψ) is a widespread RNA modification that plays an essential role in RNA stability and function. In eukaryotes, its deposition is mediated by pseudouridine synthases such as dyskerin, a core component of the H/ACA RNP complex, which is also known to contribute to telomere length regulation. While this link is well established in human biology and disease, the involvement of pseudouridylation machinery in plant telomere maintenance and physiology remains poorly understood. Because pseudouridylation occurs abundantly across many types of RNA, genes controlling this process are expected to exert broad pleiotropic effects on plant growth, development, and survival. In particular, their potential role in gametogenesis and fertility offers an intriguing direction for uncovering novel mechanisms that couple RNA modification, genome stability, and reproductive success in plants.

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

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Transcription factors (TFs) are key regulators of gene expression and plant development. The plant-specific PHD-HD TF family, defined by a plant homeodomain (PHD) and homeodomain (HD) architecture, is represented in a model plant *Arabidopsis thaliana* by only two members: HAT3.1 and PRHA. Although closely related, these proteins differ markedly in molecular features, nuclear organization, and developmental roles. HAT3.1 forms distinct nuclear condensates that partially colocalize with chromocenters, densely packed regions of constitutive heterochromatin that are typically transcriptionally inactive. A protein-interaction screen identified methyl-CpG-binding domain proteins (MBD1/2/4) as top interactors of HAT3.1; these proteins also co-localize with HAT3.1 speckles, suggesting they function together at shared nuclear sites. A histone-binding assay further showed that the PHD finger of HAT3.1 recognizes histone H4 lysine 20 trimethylation (H4K20me3), which role in plants is still largely unexplored. These features point to HAT3.1 acting within organized chromatin environments to tune gene activity. By contrast, PRHA is evenly distributed in the nucleus, and its PHD finger does not show a clear histone preference. Transgenic reporter lines also reveal distinct expression patterns: HAT3.1 is active broadly across the root meristem, whereas PRHA is mostly confined to stem-cell initials and pericycle cells. Both genes are upregulated during callus formation and shoot regeneration, key phases when cells are reprogrammed. Notably, *prha* mutants form larger callus and regenerate more efficiently, indicating that PRHA normally restrains cell proliferation and reprogramming. Together, these results reveal pronounced functional divergence within the PHD-HD family: HAT3.1 likely helps organize chromatin-based control of genes, while PRHA acts as a brake during developmental reprogramming. This information advances understanding of the largely unexplored PHD-HD TF family in plants and its roles in growth and development.

Acknowledgement
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RHBDL4 Controls B Cell Numbers via Lipids

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Rhomboid proteases are transmembrane enzymes present in almost all cellular membrane compartments and are widespread across the tree of life. They mediate cleavage of transmembrane substrates by their catalytic serine in the active site immersed inside the lipid bilayer. Such ubiquitous presence and conservation among organisms indicate important cellular functions. Paradoxically, the physiological functions of most mammalian rhomboid proteins remain poorly understood. In cases where their functions are known, such as the mitochondrial rhomboid PARL[1], they rely on unexpected biological mechanisms and are widely impactful and disease-relevant[2].

One of the mammalian rhomboid proteases is the endoplasmic reticulum (ER)-localised RHBDL4. RHBDL4 has been implicated in ER-associated degradation (ERAD)[3], ER morphology[4], lipid metabolism (SREBP1 signaling)[5], and trafficking (TMED7, TGFα)[6,7], and many studies have reported RHBDL4 upregulation in cancer cells[8]. However, its organismal role under unchallenged conditions remains largely unexplored, and the *in vivo* relevance of the published data has yet to be clarified. We aim to understand the physiological function(s) of RHBDL4 by combining findings at the cellular and organismal level. Mice deficient in RHBDL4 were studied under unchallenged conditions as well as after high-fat diet administration and antigenic stimulation. As a cellular model, we isolated and immortalized murine macrophages, thereby working with almost primary cells that were genetically modifiable. For *ex vivo* cellular studies, we isolated primary splenic B cells using fluorescence-activated cell sorting (FACS) or negative magnetic cell sorting.

Unchallenged RHBDL4-deficient mice exhibit an immune cell phenotype, primarily affecting B cell numbers (decreased by almost one-third in blood compared to wild-type). B cell functions, such as antibody production and cell motility, are almost unaffected. Surprisingly, the B-cell phenotype is rescued by administration of high-fat diet, indicating connection of RHBDL4 to lipid metabolism. Mouse chimeric experiments indicate that the decrease in B cells results from a combination of B cell intrinsic factors and external influences from hematopoietic cells, and proteomics experiments indicate influence of RHBDL4 on proteostasis of lipid biosynthetic enzymes. We currently focus on clarifying and integrating how RHBDL4 influences lipid metabolism in immune cells (our findings suggest an SREBP1 independent effect) and the link between lipids and the decrease in B cells.



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Poster Presentations

Computer simulations of sugars: are they tricky?

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Sugars, omnipresent in our daily lives, play far more than a culinary role: they are essential building blocks in biological systems. In structural biology, these sugars are mainly known as *glycans*, and they are deeply involved in cellular processes such as cell signaling and molecular recognition. Yet, compared to proteins, glycans have historically received less attention. This imbalance is largely due to a relatively limited toolset available for probing their complex structure–function relationships, which has often led to their biological significance being underestimated or even overlooked.
I will demonstrate how molecular simulations, an integral part of my research, can be used to illuminate the behavior of glycans *in silico*. Drawing on examples from my own work, I will show how simulations provide valuable predictions about glycan behavior, highlight how their unique chemical structures underpin both their functional diversity and the challenges in studying them, and illustrate how computational approaches can help overcome these challenges.

Library-based Approach for Profiling of Sequence Preferences of Rhomboid Proteases

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Rhomboid intramembrane serine proteases are conserved across all domains of life, where they fulfill different functions ranging from bacterial quorum sensing to the regulation of developmental signaling in *Drosophila*[1]. In mammalian cells, they regulate processes such as mitophagy, apoptosis, epithelial tissue maintenance, and mitochondrial homeostasis. Dysregulation of rhomboid activity has been linked to multiple diseases, including Parkinson's (PARL) and Alzheimer's (RHBDL4), type II diabetes (PARL), as well as epithelial (RHBDL2) and colorectal cancers RHBDL4)[2-3]. These associations highlight rhomboid proteases as attractive, yet underexplored, therapeutic targets.

The most advanced rhomboid inhibitors to date are based on the α-ketoamide skeleton with oligopeptide substrate-derived 'head' and hydrophobic 'tail'. Both 'heads' and 'tails' can be subject to tailoring, which has been shown to influence the specificity and potency[4-6]. However, progress in inhibitor development is hindered by the lack of efficient methods to systematically probe rhomboid substrate preferences.

Here, we present a library-based fluorescence assay that enables rapid mapping of rhomboid substrate sequence preferences using fluorogenic peptide substrates. We validated this approach on the bacterial rhomboid protease GlpG and the human protease RHBDL2. The identified sequence preferences were then applied to optimize α-ketoamide inhibitors, yielding improved potency. By linking substrate preferences to inhibitor design, this approach advances the development of selective rhomboid inhibitors with therapeutic potential.

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Click Chemistry Tools for Cell Labeling and Small Molecule Targeting

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Targeting small molecules to specific organelles holds significant potential for medical applications. Various strategies focus on delivering active drugs or fluorophores to intracellular compartments. Here, we exploit the bioorthogonal click chemistry reaction between trans-cyclooctene (TCO) and 1,2,4,5-tetrazine (Tz) to covalently link organelle-targeting probes with fluorophores. To achieve this, the U2OS cells were sequentially incubated with organelle-targeting probes and fluorophores containing complementary click reaction partners. Colocalization of the click products with organelle-specific markers was evaluated using confocal microscopy and image analysis. Selected organelle-targeting probes demonstrated specific localization to the plasma membrane (PM), nucleus, endoplasmic reticulum (ER), lysosomes, and mitochondria (Figure 1). Our findings demonstrate that the TCO-Tz click reaction enables targeted delivery of molecular cargos to specific organelles. This reaction can alter cargo properties, facilitating changes in intracellular distribution. Bioorthogonal reactions may be a new means for more precise medicine. This approach may enhance drug delivery efficiency, minimize off-target effects, and enable controlled therapeutic interventions. Future studies could further refine the selectivity and efficiency of these reactions, broadening their potential applications in precision medicine and intracellular imaging.

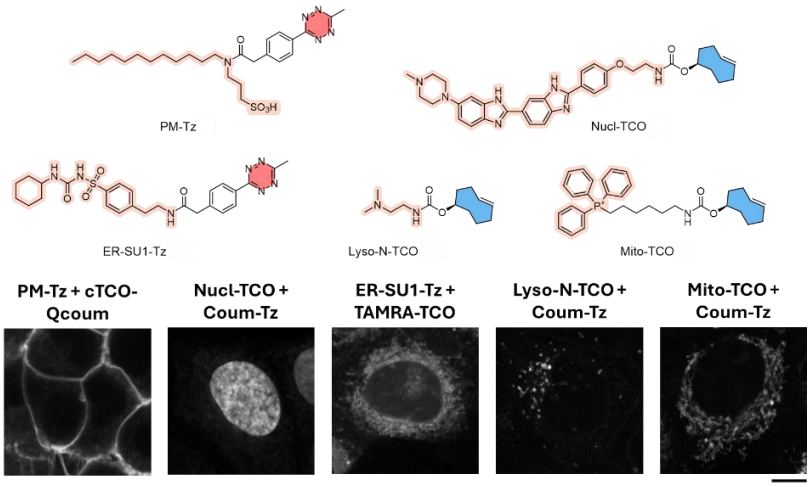


Figure 1. The structures of the organelle-targeting probes and their cellular localization. The organelle-targeting groups are highlighted in pink, trans-cyclooctene (TCO) in blue, and tetrazine (Tz) in red. Scale bar 10 μm

This work was supported by the project New Technologies for Translational Research in Pharmaceutical Sciences /NETPHARM, project ID CZ.02.01.01/00/22_008/0004607, co-funded by the European Union, and National Institute for Metabolic and Cardiovascular Disease Research (Programme EXCELES, ID Project No. LX22NPO5104) - Funded by the European Union - Next Generation EU.

Charge Scaling Force Field for Biologically Relevant Ions Utilizing a Global Optimization Method

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Classical fixed-charge force fields, while widely used in molecular simulations, often struggle to accurately capture ion–water and interionic interactions[1]. Polarizable force fields provide a more rigorous treatment but remain impractical for complex systems due to their substantially higher computational cost[2]. Charge scaling, also known as the electronic continuum correction (ECC)[3], offers an efficient alternative by implicitly incorporating electronic polarization into classical simulations without added expense. Charge scaling, also denoted as the electronic continuum correction, has proven to be an efficient method for effectively including electronic polarization in force field molecular dynamics simulations without additional computational costs. However, scaling charges in existing force fields, fitted at least in part to experimental data, lead to inconsistencies, such as overscaling. We have, therefore, recently developed a four-site water model consistent with charge scaling, i.e., possessing the correct low-frequency dielectric constant of 45[4]. Here, we build on top of this water model to develop charge-scaled models of biologically relevant ions, denoted as ECCions81, including Li^+, Na^+, K^+, Ca^{2+}, and Mg^{2+} cations as well as Cl^-, Br^-, and I^- anions. Parametrization was streamlined and accelerated using a machine learning–assisted optimization framework. On the one hand, the resulting models outperform the best existing ECC-based ion models in reproducing structural, transport, and dynamical properties of electrolyte solutions, with only a minor compromise in bulk density predictions. On the other hand, the present work points to a future need for consistently and simultaneously improving the water and ion models within the electronic continuum correction framework.

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Structural insights into how bacteria regulate the expression of carbon-catabolism enzymes

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The process of the gene expression comprises number of steps, including transcription of DNA into RNA, translation of RNA into protein, and protein modifications. In bacteria, the regulation of the expression pathway represents the major answer to any environmental changes, therefore a critical adaption for cell survival. Based on the availability of different nutrients, bacteria turns on and off different genes encoding for proteins of the metabolic pathways. The whole metabolic machinery can be regulated at the very beginning of the expression, namely the initiation of transcription. For that, diverse bacterial groups use transcription repressors of the GntR family. The members of the GntR family are mainly responsible for regulation of enzymes utilizing catabolism of various carbon sources (e.g. sugars). The family is defined by a conserved N-terminal DNA-binding domain (DBD) with winged-helix-turn-helix motif. The C-terminal effector binding domain (EBD) distinguishes these regulators into five subfamilies. When an effector binds the EBD, the transcription repressor loses its ability to bind DNA, which consequently leads to activation of transcription. Although there are thousands of these repressors across most bacterial species, their mechanism of action is hardly ever understood on the structural level. Here we present the initial structural biology study on the prototypic member GntR, from the model organism of gram-positive bacteria *Bacillus subtilis*. By the method of cryogenic electron microscopy (cryoEM), we try to understand the molecular mechanism behind the protein-DNA complex formation, what are the spatial arrangements, and how these change upon effector binding.

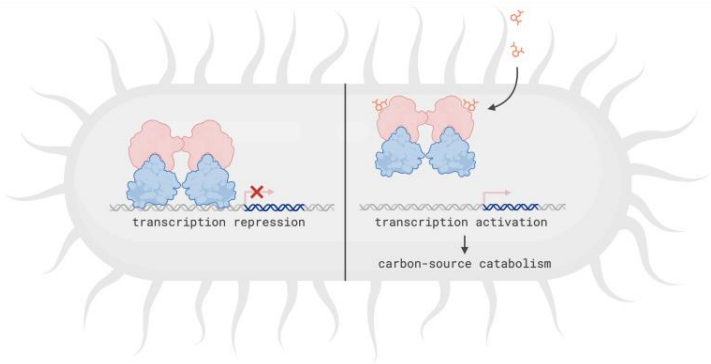


Figure 1. Schematic overview of the regulation of gene expression at the step of transcription initiation in bacteria. When the nutrient effector molecule is not present in the environment, the transcription repressor of GntR family binds to DNA and turns off expression of the proteins involved in the nutrient metabolism. When the carbon-source nutrient is available, it is transported into the cell, binds the transcription repressor and inhibit its capability of DNA binding, leading to transcription activation and consequent expression of the carbon-source catabolism machinery.

Editing-independent effects of Adar in *Drosophila melanogaster*

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Vertebrate ADAR RNA editing enzymes prevent cellular dsRNA from aberrantly activating antiviral dsRNA sensors. ADARs inhibit antiviral sensor activation by deaminating selected adenosines to inosines in dsRNA and by engaging in inhibitory protein interactions with sensors on dsRNA. ADARs interact with Dicers and, in the *Drosophila Adar*^{5G1} null mutant Dicer 2 acts as the antiviral dsRNA sensor mediating aberrant innate immune induction. We overexpressed active Adar isoforms or a catalytically-inactive Adar E374A protein from *UAS-Adar* constructs under the control of a temperature-regulated *Act5C*^{ts}-*GAL4* driver. Overexpression of the edited AdarG isoform or AdarEA cause larval lethality with aberrant innate immune induction. Some escaper pupae are formed with head eversion defects.. Ecdysone signaling gene and innate immune gene expression are aberrantly elevated in the AdarG overexpressing pupae. RNAi knockdown of *Ecdysone Receptor A* (*EcR A*) or increasing expression of HP1 partially rescue AdarG overexpression defects and normalize gene expression in progeny flies, indicating that aberrant epigenetic silencing is also involved.

Design, synthesis and biological evaluation of inubosin-like compounds as Ngn2 promoter activators

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The induction of neurogenesis is believed to be a therapeutic strategy for neurodegenerative diseases and neuronal injuries. Neurogenin 2 (Ngn2) is one of the key regulators of neurogenesis. The use of Ngn2 promoter activator aids neurogenesis induction. Inubosin B – acridine alkaloid– can start regeneration of neurons via activation of Ngn2 expression. Here, we prepared a series of *N*-alkyl-4-methoxyacridine-2-carboxamides inspired by the inubosin B scaffold. At 5 μM concentration, compounds, **1, 7k**, and **7l** were more active than the parent compound inubosin B and formononetin – the most active non-glycosylated Ngn2 promoter-activator reported so far. Additionally, these compounds had low cytotoxic effect on the reporter cell line (C3H10T1/2) and HepG2 cell line.[1,2]

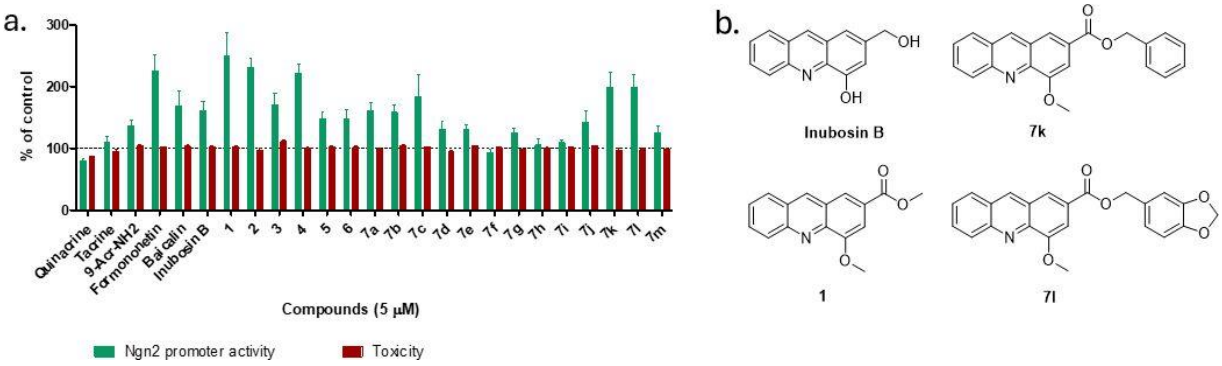


Figure 1. a. Ngn2 promoter activity for inubosin-like compounds and their toxicity on C3H10T1/2 cell line (24 h treatment) at concentration of 5 μM; **b**. the structures of the most active compounds and inubosin B

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Basal Ganglia Neurons in Alzheimer's Disease and Post-Stroke Dementias

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Dementia is a debilitating syndrome of cognitive impairment with risk factors including older age, and vascular and neurodegenerative pathologies. The basal ganglia containing the putamen are subcortical nuclei particularly vulnerable in dementia involved in motor, cognitive, and emotional processing.[1] The putamen is composed mainly of inhibitory gamma-aminobutyric acid (GABA) medium-sized spiny neurons, and cholinergic and GABAergic interneurons. Stereology is a reliable technique for determining quantitative changes in neuronal morphology based on unbiased random sampling.[2] Using unbiased 3D stereology on cresyl-fast violet-stained coronal brain tissue sections from 54 elderly post-stroke (non-) demented [PS(N)D] or Alzheimer's disease (AD) patients, the aim was to evaluate neuronal densities and volumes in the putamen. The observed neurons shared small size, suggesting large neuron shrinking to drive grey matter loss but retaining neuronal densities. Neuronal densities did not vary significantly across disease groups unlike volumes, where AD had significantly shrunk neurons compared to PS(N)D consistent with strong AD neurodegeneration associations. Control was unexpectedly insignificantly different from other groups likely due to disproportionate analyses in the pre- or post-commissural putamen. There were significant negative correlations between densities and volumes in most groups consistent with previously published reports suggesting compensation for decreasing neuronal densities with increasing volumes. The average neuron density was estimated to be roughly 1×105 cells/mm3 with an average volume of 530 μm3. Follow-up stereology analyses using automated approaches, employing more human subjects and utilising mainly immunohistochemical stains were identified as opportunities for future work.

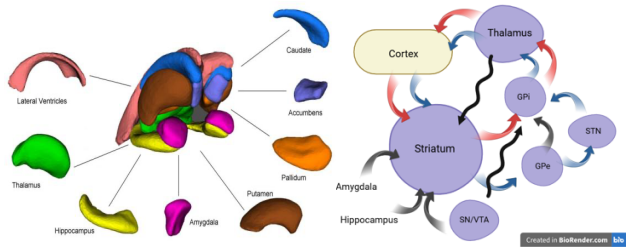


Figure 1. Basal ganglia schematic (left) and corticostriatal circuitry (right). Red arrows – direct pathway, blue – indirect, black – other. Abbreviations: GPi = internal segment of globus pallidus, GPe = external segment of globus pallidus, SN = substantia nigra, STN = subthalamic nucleus, VTA = ventral tegmental area. Adapted from Goodro et al. 2012 (3) and Haber et al. 2016 (4). Made in Biorender.com..

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Design of Fusogenic Peptides

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Efficient intracellular drug delivery remains a major challenge in modern medicine, largely due to the difficulty of crossing cellular membranes and inefficient endosomal escape,[1] both of which stem from the lack of a reliable strategy for site-specific membrane fusion. Viruses overcome these obstacles using specialized fusion proteins,[2] inspiring the development of synthetic fusogenic peptides; however, natural fusion peptides lack specificity and are difficult to adapt for therapeutic use, underscoring the need for rationally designed alternatives. This project will apply computational methods to identify peptide motifs that regulate distinct steps of membrane fusion, enabling the rational design of synthetic fusogenic peptides with tailored activity. The designed peptides will then be validated through experimental assays assessing both fusogenic activity and cargo delivery capacity. The expected outcomes include a mechanistic understanding of how sequence motifs govern membrane fusion, a library of synthetic peptides with tunable activity, and initial validation of their potential for drug delivery applications. Beyond oncology, these peptides could provide a versatile platform for gene- and RNA-based therapeutics as well as treatments for neurological and autoimmune diseases.

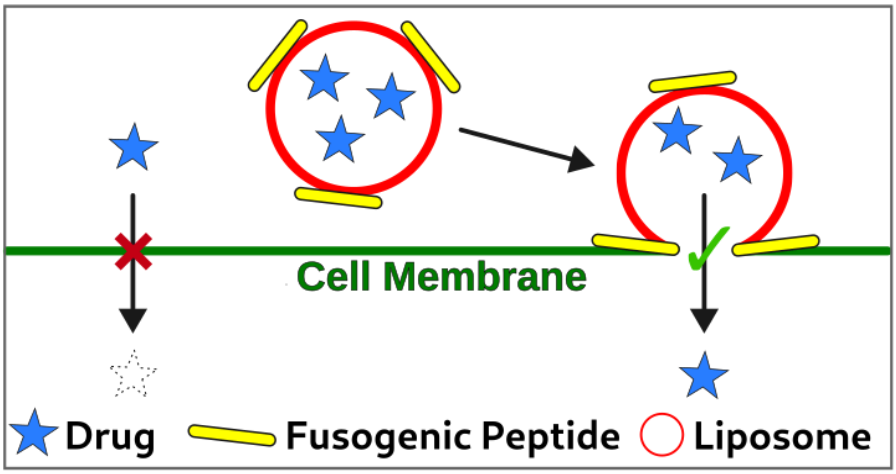


Figure 1. Fusogenic peptides enable the delivery of membrane-impermeable drugs

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Histone chaperones ASF1 and HIRA regulate telomere length and 45S rDNA stability in *Arabidopsis thaliana*

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Genome stability relies on the proper organization of chromatin, the DNA–protein complex that forms chromosomes. For the genome to function and be faithfully replicated, histones must be correctly assembled and removed during processes such as DNA replication, transcription, and repair. These tasks are facilitated by histone chaperones, specialized proteins that assist in histone handling.
In this work, we investigate two histone chaperones in the model plant *Arabidopsis thaliana*: ANTI-SILENCING FUNCTION 1 (ASF1) and HISTONE REGULATOR A (HIRA). We focus on their roles in maintaining telomeres — the natural ends of chromosomes — and the 45S ribosomal DNA (rDNA) loci, which encode the RNA components of ribosomes. Both regions are highly repetitive and particularly sensitive to changes in chromatin structure.
We found that ASF1 and HIRA are essential for telomere length regulation. In mutant plants lacking these genes, telomeres are significantly shorter, yet they still maintain nucleolar localization and histone H3 occupancy comparable to wild-type telomeres. Beyond telomere regulation, ASF1 and HIRA also contribute to the silencing of the 45S rRNA genes and affect their copy number. ASF1 further contributes to global heterochromatin maintenance. Finally, we show that ASF1 transiently physically interacts with proteins directly involved in telomere biology, including TELOMERE REPEAT BINDING 1 and the N-terminal domain of telomerase.
Altogether, our findings suggest that histone chaperones ASF1 and HIRA contribute to genome stability and may support the proper function of telomeres and rDNA.

Mild C–F activation of perfluoroalkyl substances

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Perfluoroalkyl substances (PFAS) are a large group of synthetic compounds widely used in textiles, coatings, and firefighting foams. Their strong carbon–fluorine bonds make them extremely resistant to breakdown, leading to their accumulation in water, soil, and living organisms. Because of this persistence, PFAS are often referred to as “forever chemicals” and represent a serious global environmental challenge.[1] Current strategies for their removal, including incineration, adsorption, and electrochemical treatments, are costly, difficult to scale, and can generate harmful by-products.[2]

In this study, we present a new chemical approach to transform PFAS derivatives under mild and selective conditions. The method is based on the synthesis of perfluoroalkyl azides, which act as stable intermediates. These intermediates allow the elimination of fluoride and create new reactive sites that can be converted into nitrogen- and sulfur-containing organic molecules. Importantly, the reactions proceed without the need for metal catalysts or external energy input, and they tolerate a wide range of functional groups.

Mechanistic studies, supported by spectroscopy, X-ray analysis, and computational modeling reveal the formation of key intermediates that explain how the transformation proceeds.[3] This work demonstrates a practical and sustainable route for turning persistent fluorocarbons into useful chemical building blocks, highlighting how fundamental chemistry can contribute to addressing urgent environmental problems.

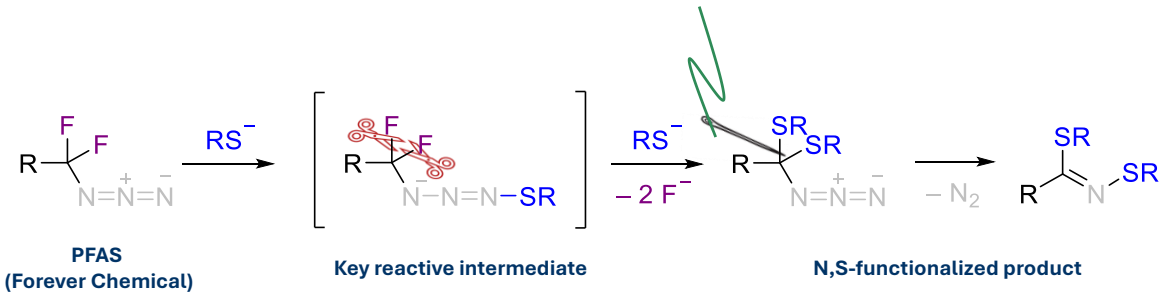


Figure 1. A simple reaction turns stable, toxic PFAS into nitrogen- and sulfur-containing products under mild, metal-free conditions.

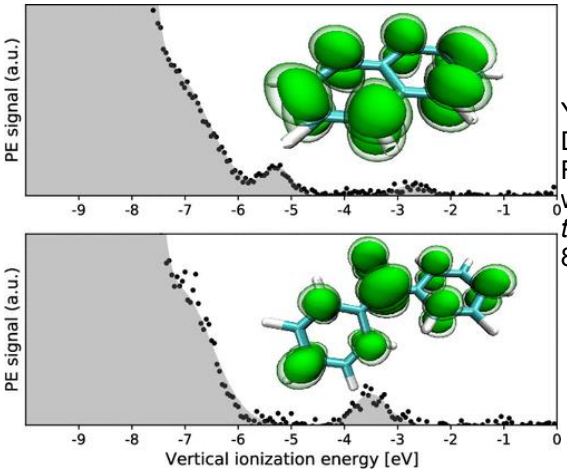
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Stability and Reactivity of Aromatic Radical Anions with Relevance to Birch Reduction

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Aromatic radical anions are key intermediates in many chemical processes, including Birch reduction, a well-established method for hydrogenating aromatic hydrocarbons. We have characterized these intermediates both theoretically and experimentally, focusing on their electronic structure and reactivity. Using synchrotron photoelectron spectroscopy and cyclic voltammetry, we determine their electron binding energies and redox potentials, with electronic structure calculations confirming agreement with experimental values, clarifying the Birch reduction mechanism. Expanding beyond benzene, we investigate the radical anions of naphthalene and benzophenone using liquid-jet photoelectron spectroscopy and electronic structure methods. Our findings not only provide new insights into the stability and electronic properties of these radical anions in solution but also emphasize the crucial role of solvent choice in optimizing Birch reduction conditions. Furthermore, this work highlights the importance of theoretical calculations in bridging distant experimental techniques and lays the groundwork for developing alternative solvents for Birch-like reactions.



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Uncovering Ap2N-Capped Human tRNAs and Their Fragments with Ap2N-RNA Sequencing

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It was long believed that the eukaryotic RNA was only capped with 7-methylguanosine. However, the expanding field of non-canonical RNA caps recently revealed the presence of other structures (nicotinamide adenine dinucleotide, flavin adenine dinucleotide, dinucleoside polyphosphates) at the 5' end of various mRNAs and non-coding RNAs[1-3]. We report here on the discovery and quantification of a new type of RNA cap - adenosine nucleoside diphosphate (Ap_2N) - on mammalian RNA. We established an LC-MS method to measure Ap_2N-RNA levels and observed its increase under oxidative stress. To identify Ap_2N-capped RNAs, we have developed a new sequencing method (Ap_2N-RNA Seq) by leveraging the ability of truncated T4 RNA Ligase 2 to selectively ligate Ap_2N-capped RNAs to an RNA adaptor. By applying this method to human short RNA, we have found a subset of tRNAs capped with Ap_2N. Surprisingly, in some cases, we observed Ap_2A at the 5′ end of yet not described 3′ tRNA fragments. The presence of Ap_2A at the 5' termini of selected hits was further confirmed by orthogonal methods. This report presents evidence of a previously unknown type of tRNA capping and processing. Our methodology creates new opportunities for studying the intricate processes of the tRNA life cycle.

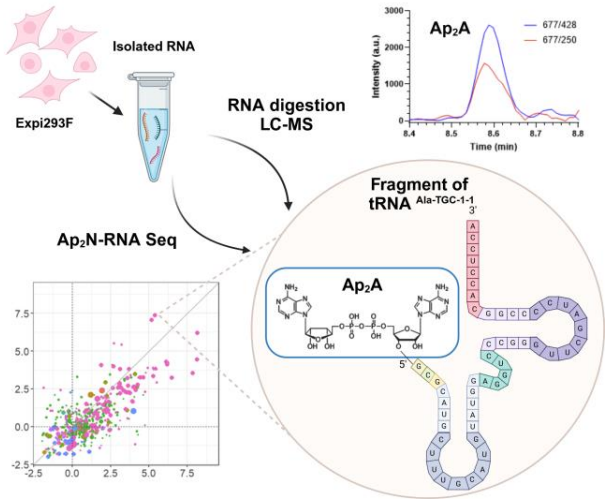


Figure 1. RNA isolated from Expi293F cells was either digested and analysed using LC-MS in order to quantify Ap_2N RNA cap or subjected to Ap_2N-RNA Seq that lead to identification of Ap2N-capped RNAs including new previously unknown tRNA fragments.

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Proteomic Analysis of Histone Post-Translational Modifications and Mapping of Histones in Human Sperm

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Epigenetics is a rapidly expanding field of biology that explores changes in gene expression without alterations to the underlying DNA sequence. One of the key regulatory mechanisms involves post-translational modifications (PTMs) of histones – proteins around which DNA is tightly coiled. Research revealed a broad spectrum of histones PTMs such as acetylation, methylation, phosphorylation, and the importance of their combinations. Together, these chemical marks constitute the "histone code," a complex regulatory language that precisely fine-tunes chromatin structure and cellular function.

In human health, histone PTMs are increasingly recognized as valuable biomarkers across a range of diseases. For instance, reduced acetylation of lysine (K) at specific sites on the H4 histone, such as H4K16ac and H4K20ac, has been associated with tumor progression and poor prognosis in several cancer types[1]. Such findings have driven the development of histone deacetylase inhibitors, some of which have already been approved by the U.S. Food and Drug Administration for clinical use[2]. Beyond medicine, epigenetic regulation plays a crucial role in plant biology, influencing development, stress response, and crop productivity. Thus, accurate mapping of histone PTMs is essential not only for understanding gene regulation and disease mechanisms but also for unlocking future applications in medicine and sustainable agriculture.

Mass spectrometry has become the gold standard for mapping and quantifying histone peptidoforms. This approach provides exceptional sensitivity and resolution, allowing researchers to unravel the complexity of the epigenetic landscape. This poster presents a concise overview of the proteomic workflow for histone PTM analysis using mass spectrometry and highlights a case study demonstrating the clinical potential of histone proteomics in human sperm, offering novel insights into the molecular basis of male infertility.

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Correlative Fluorescence and Cryo-electron Microscopy for Biofilm Matrix Ultrastructure Analysis

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Bacterial biofilms are a leading cause of persistent infections, particularly in clinical contexts involving implanted medical devices. Their resistance to antibiotics and host defenses presents a major challenge to healthcare systems worldwide[1,2]. This project, currently in the stage of proposal for an MSCA fellowship, aims to integrate advanced cryogenic electron tomography (cryo-ET), correlative fluorescence microscopy, and state-of-the-art image analysis pipelines to resolve the structural principles that underlie biofilm resilience at the micrometer scale.
The research combines in vivo metabolic labelling, high-pressure freezing, and cryo-focused ion beam milling to generate vitrified samples of biofilm communities in their near-native state. Correlative cryo-fluorescence and cryo-electron microscopy enables precise localization of fluorescent markers within biofilm matrices, while subtomogram averaging and computational workflows reveal macromolecular assemblies contributing to antimicrobial tolerance.
The results are expected to provide fundamental insights into the architecture and function of bacterial biofilms, opening avenues for the development of diagnostic tools and personalized therapeutic strategies[3]. Beyond clinical relevance, the methodological advances—particularly the cryo-compatible fluorescent marker panel and correlative imaging pipeline—will be broadly applicable to diverse research areas in cell biology and microbiology.
Overall, the project highlights the power of integrative cryo-imaging approaches for tackling global health challenges such as antimicrobial resistance, aligning with European and international priorities to improve infection management and reduce the burden of chronic disease.

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Decoding *Trifolium* Chromosomes: Oligo-FISH Mapping of Karyotype Evolution

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The genus *Trifolium* (clover), one of the largest in the legume family, includes over 250 species that are ecologically and agriculturally significant, particularly as forage crops. Despite their importance, the evolutionary dynamics of *Trifolium* genomes remain poorly understood, especially in terms of chromosome rearrangements and dysploidy. Notably, *T. pratense* (red clover) has a reduced chromosome number ($2n = 14$) compared to the ancestral state ($2n = 16$) suggesting descending dysploidy and complex karyotype evolution. This study aims to elucidate the chromosomal mechanisms underlying genome evolution in *Trifolium*, focusing on the transition from the ancestral to the derived karyotype. We ask: What chromosomal rearrangements accompanied the reduction in chromosome number in the genus *Trifolium*? Can these rearrangements inform about phylogenetic relationships among closely related species?

To address these questions, we developed and optimized a high-resolution oligo-FISH methodology using chromosome-specific probes designed from the reference genome of *T. pratense*. Using this approach, we investigated chromosome structure in *T. pratense* ($2n = 14$), *T. pallidum* ($2n = 16$), and *T. ochroleucon* ($2n = 16, 32$). Preliminary results reveal distinct patterns of chromosomal rearrangements, including fusions and translocations. These rearrangements also provide cytogenetic evidence supporting or refining phylogenetic relationships among the studied species.

Our findings demonstrate the power of oligo-FISH painting in resolving fine-scale chromosomal changes and offer new insights into the evolutionary history of *Trifolium*. By integrating cytogenomic tools with comparative cytogenetics, this work advances our understanding of genome evolution in legumes and sets the stage for broader applications in plant systematics and breeding.

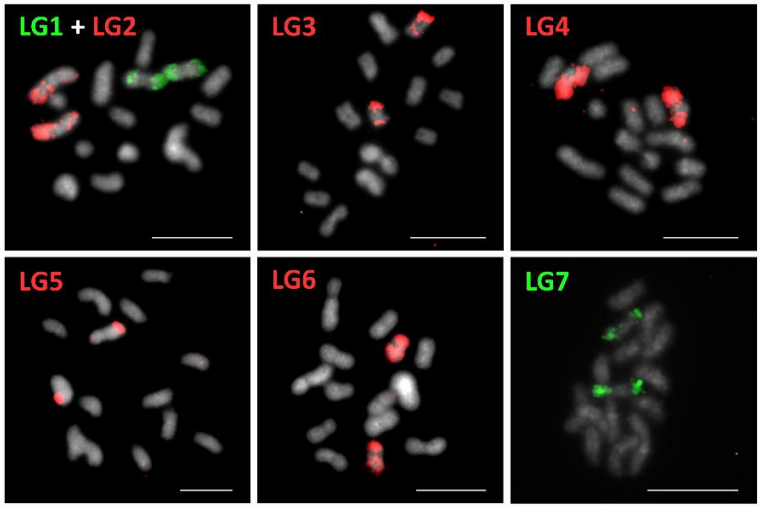


Figure 1. Fluorescence *in situ* hybridization of oligo-FISH probes corresponding to linkage groups LG1 to LG7 on mitotic chromosomes of reference species *Trifolium pratense*. Chromosomes were counterstained by DAPI. Scale bar: 10 µm.

Telomerase RNP complex in *Arabidopsis thaliana*: impact of the components on telomerase activity and telomeres

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Telomerase is a ribonucleoprotein complex (RNP) responsible for maintaining the integrity of telomeres, nucleoprotein structures at the end of eukaryotic chromosomes. Telomerase comprises two main subunits, telomerase reverse transcriptase (TERT) with its catalytic activity and telomerase RNA (TR), which provides an RNA template for the synthesis of telomeric DNA repeats by TERT, and is highly divergent in length, sequence and biogenesis across different species. The description of *A. thaliana* telomerase RNP complex, its regulation and role of its components is currently quite limited. We decided to utilize *A. thaliana* dyskerin homologue, which is as well part of a complex responsible for RNA pseudouridylation, and identify its interaction partners by co-purification. Further, we examined the heterozygous or homozygous mutants of presumed RNP complex components in terms of their effects on telomerase activity, telomere length and *TR* and *TERT* transcription, to evaluate their roles in telomerase function and regulation.

Interactions of CD44 with TLR4-MD-2 complex and it's influence on hyaluronan binding

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Toll-like receptors (TLRs) are widely distributed in the gastrointestinal tract as part of the innate immune system, making them relevant in the treatment of carcinomas of the duodenum, small intestine, colon, and rectum. Short hyaluronic acid (HA) oligosaccharides activate signaling cascades upon binding to TLR4, leading to the production of angiogenic cytokines IL-1β and IL-8. HA plays a key role in drug resistance mechanisms, as cancerous cells exhibit a higher proportion of low molecular weight hyaluronan (LMW-HA) in the extracellular matrix (ECM). This triggers pathways responsible for ECM remodeling and promotes tumor cell aggressiveness.
Immunoprecipitation studies have shown that CD44 and TLR4 can physically associate. Interestingly, CD44-knockout cells still exhibit HA binding to TLR4, indicating that TLR4-induced pathways are CD44-independent but benefit significantly from CD44 presence. We propose three hypotheses regarding how CD44 assists HA binding, which require further molecular dynamics simulations for confirmation: (1) CD44 blocks HA from interacting with off-target regions of the TLR4-MD-2 complex, (2) CD44 actively binds HA, limiting its degrees of freedom and directing it to the TLR4-MD-2 binding site, and (3) CD44 increases the local effective concentration of HA around the TLR4-MD-2 complex.
In this work, we use enhanced sampling molecular dynamics simulations to find optimal structure of the CD44-TLR4-MD-2 complex. Our model incorporates existing glycosylation data[1,2,3] for these proteins, which limits their conformational states. The placement of HA-binding residues of CD44 in proximity to TLR4-MD-2 suggests that CD44 acts as a facilitator by maintaining HA close to the complex, thereby increasing the likelihood of cascade activation. Found complex structure is used to assess differences in binding HA by TLR4-MD-2 with and without presence of CD44.

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PhiKZ Baseplate Structure

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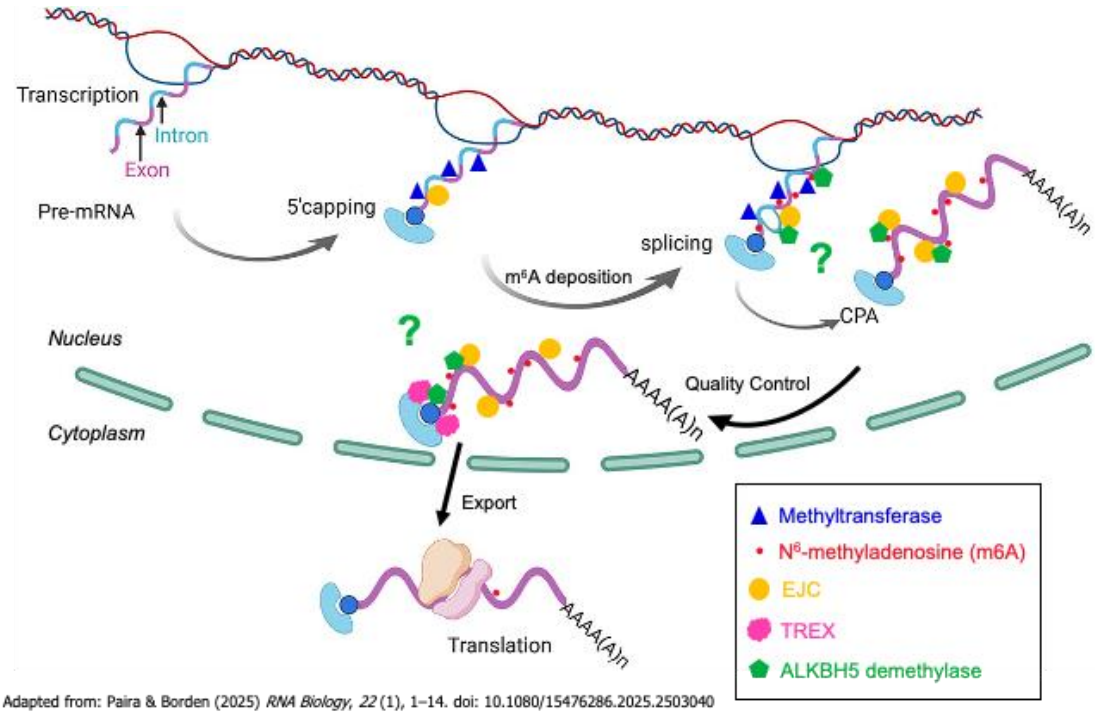
PhiKZ is a bacteriophage that infects Pseudomonas aeruginosa, an opportunistic human pathogen. The phage phiKZ is known for its large genome and complex structure, making it a notable subject for struc-tural biology studies. Among all its parts, the baseplate is the most complex. It adheres to the phage prey and triggers the genome ejection. The signal that triggers the ejection travels from the tail fibres to the tail through the baseplate. Here, we used cryo-Electron Microscopy to visualise the structure of the phiKZ tail and the baseplate at high resolution, the first one of a jumbo bacteriophage. Our results reveal an intricate network of proteins organised in six-fold symmetry. Structural comparisons with related sys-tems highlight the universal conservation observed in contractile injection systems. This sheds light on phiKZ's baseplate specificities and suggests a potential mode of action. Its structural analysis enhances our understanding of phiKZ and contributes with valuable knowledge to the broader field of myovirus biology.

Uncovering the mechanisms of ALKBH5 demethylase in RNA biogenesis

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The N6-methyladenosine (m^6A) RNA modifications, the crucial layer of epitranscriptome regulation implicated in various human diseases, are balanced by methyltransferases (MTs) and demethylases (DMTs) activities. Dysregulation of ALKBH5, one of two known human DMTs, has been linked to defects in multiple stages of mRNA biogenesis linked to the development of acute myeloid leukemia (AML) and colorectal cancer. It has been proposed that the role of ALKBH5 in poor cancer prognosis relies in impairment of m^6A levels, and degradation of target mRNAs of cancer-modulating genes. However, recent findings have revealed that m^6A abundance is primarily regulated by splicing efficiency and exon-junction complex (EJC) deposition rather than ALKBH5 activity. The proposed project aims to uncover the molecular mechanisms of ALKBH5 protein in mRNA biogenesis and quality control. By utilizing the state-of-the-art methods and pipelines to identify ALKBH5-dependent m^6A deposition and mRNA-protein interactions throughout the mRNA biogenesis, such as the miCLIPseq and PAR-CLIP, we will address the role of ALKBH5 in recruitment of modifying factors to mRNA and its impact on m^6A profile. For this purpose, HEK293T cells, with endogenously tagged *ALKBH5* and *ALKBH5*-KO, will be used.



Adapted from: Paira & Borden (2025) *RNA Biology*, *22* (1), 1–14. doi: 10.1080/15476286.2025.2503040

Kmerth: A Kmer-Based Pipeline for Unique gRNA and FISH Discovery from Chromosomal-Level Genomes

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Introduction: Identifying reliable guide RNAs (gRNAs) for CRISPR-based genome editing and designing fluorescent *in situ* hybridization (FISH) probes for cytogenetic visualization remain challenging, especially in large, repetitive genomes. To address this, we developed Kmerth, a bioinformatic pipeline that systematically identifies unique gRNA and FISH targets from chromosomal-level genome assemblies.

Objectives: Kmerth was designed to: (1) Detect unique genomic sequences suitable for FISH probe design. (2) Discover high-confidence gRNA candidates for Cas9 and Cas12 systems. (3) Provide a unified, computational efficiency workflow that ensures high target specificity.

Methods: Kmerth accepts chromosomal-level genome assemblies and allows users to define regions of interest. These regions are separated from the rest of the genome and treated as distinct sets. Kmer analysis is performed using either *jellyfish* or *meryl*, with user-defined kmer sizes. Unique kmers that are present only in the target region and absent elsewhere are retained and mapped back to the genome using *BWA*, *minimap2* and *BLAST*, applying an 80% similarity threshold to eliminate non-unique hits. For each remaining k-mer seed, Kmerth retrieves the surrounding sequence from the original genome assembly to construct candidate loci. Each locus is then greedily extended on both sides, while uniqueness is continuously verified by mapping against the rest of the genome using *BWA*, *minimap2* and *BLAST*. Extension halts when user-defined length is reached or further elongation introduces non-unique matches. The longest unique sequences are retained and clustered by sequence similarity. Potential gRNA targets are identified by scanning for Cas9 and Cas12 PAM sites. These are further filtered through remapping and similarity screening. The final set of unique sequences with PAM sites is prioritized based on copy number and experimental suitability as FISH probes.

Results: To validate Kmerth, we applied it to chromosomal-level assemblies of *Boechera exilis* × *thompsonii* and *Boechera crandallii* × *thompsonii*. The pipeline successfully identified unique centromere-specific sequences on chromosome 1, from which gRNA candidates and FISH probes were generated. Experimental FISH confirmed the specificity and robustness of the selected probes.

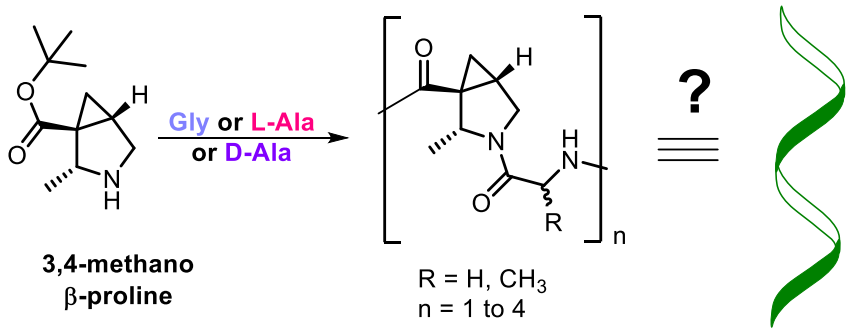
Discussion: Kmerth effectively integrates computational prediction with empirical validation, offering a versatile framework for CRISPR and FISH applications in both plant and animal genomes. Unlike existing tools that struggle with repetitive regions, Kmerth uniquely combines kmer filtering, greedy extension, and PAM site prioritization to ensure both specificity and experimental viability.

New Class of Rigidified α/β Foldamers with 3,4-Methano-β-Proline

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Polyproline can adopt two types of structures, polyproline I (PPI) a right-handed helix and polyproline II (PPII) a left-handed helix. The PPII is the structural motif of collagen, beside that it has several biological functions, and it occurs in proline-rich sequences as well as in poly(glutamic acid) and poly(alanine) sequences[1]. Several examples of synthetic polyprolines that can adopt PPI and PPII type of structures have been already reported with applications in biomaterials and collagen mimics[2].
The importance of polyproline inspired us to design and synthetize a new class of α/β foldamers composed of non-natural 3,4-methano-β-proline[3] and amino acids like glycine, L-alanine and D-alanine.
The secondary structures studies, crucial step in the field of foldamers, were performed by NMR, chiroptical methods (ECD and VCD), x-ray crystallography and computational studies.



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Enabling Next Generation Enzyme Mimics Through Local Electric Field Control in Organic Cage Catalysts

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Electric fields (EFs) have the potential to accelerate chemical reactions by stabilizing charge distributions in transition states. However, in solution, random molecular tumbling makes it difficult to align external EFs with dipolar catalysts, limiting their practical use in catalyst design. Enzymes overcome this challenge by imposing strong, oriented local fields on bound substrates, but probing these effects in complex biological systems is difficult. We present a supramolecular organic cage catalyst as a compact, tunable scaffold to study and exploit oriented EFs in well-defined cavities. Using theory, we (i) identify the applied field axis that accelerates an acyl transfer reaction inside the cage, (ii) quantify that 71% of the applied EF is directly converted into barrier reduction, and (iii) show that introducing strategically placed charged substituents generates local, through-space fields that mimic enzyme active sites. These modifications yield cavity field strengths of ~0.2 V/Å, comparable to enzymes, with a predicted 100-fold rate acceleration—achieving a 50% field-to-rate conversion efficiency. Finally, we propose a generalizable finite capacitor model to predict how local, oriented fields enhance catalysis in dipolar environments. Our results highlight supramolecular cages as promising platforms for EF-promoted catalysis and as model systems to bridge enzyme-like field effects with synthetic catalyst design.

Development of Paramagnetic NMR Spectroscopy for Structural Analysis

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Nuclear Magnetic Resonance (NMR) Spectroscopy is a ubiquitously used technique in chemistry, structural biology, material science and medical science (there known as Magnetic Resonance Imaging). Usually, the molecules studied by NMR comprise a closed-shell system. However, many transition-metal (TM) complexes (essential part of metalloenzymes, components of catalysts in chemical industry, or single-molecule magnets) have an open-shell electron configuration with one or more unpaired electrons associated with the metal atom. The presence of the unpaired electron results in long-range perturbations causing significant signal broadening and large shifts of signals (paramagnetic chemical shift). These NMR shifts report on interaction between nuclear and electron spins, which provides great detail of electronic structure of these complexes, from which stems the origin of their chemical reactivity, properties, and biological function[1].

The NMR chemical shifts can be predicted by quantum chemistry methods including density functional theory (DFT). Currently it is possible to calculate paramagnetic NMR shifts in transition-metal complexes with negligible zero-field splitting (ZFS)[2]. The problem comes for heavier-atom TM complexes (e.g. lanthanides) with non-negligible ZFS and/or low-lying exited states. The presented research circles around the development of an easy-to-implement pipeline for calculation of paramagnetic NMR shifts of ligand atoms in metal (lanthanoid) complexes with non-negligible ZFS with the use of DFT and multireference (MR) quantum chemistry calculations. Such a pipeline would be essential for the rational design of next-generation catalysts, artificial biomolecular machines, and paramagnetic tags used in structural biology.

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Simulations of resonance Raman optical activity spectroscopy for transition metal EDDS complexes

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Spectroscopy of Raman optical activity (ROA) is a chiral version of Raman spectroscopy used for studies of pharmaceutically active substances, peptides, and other biomolecules. However, the measured signal is often weak, but can be increased if the molecule significantly absorbs the exciting light, i.e. the light resonates with an electronic excited state. This technique is called resonance ROA (RROA).[1] Since it is still a relatively novel technique, a reliable simulation protocol for interpretation of experimental spectra is needed.
Here we will present experimental RROA spectra for two resonating chiral transition metal complexes (Co^{III}EDDS, Cr^{III}EDDS) supported by quantum-chemically simulated spectra. The Raman and ROA transition intensities were computed with a vibronic path-integral approach[2, 3] including several resonating excited states. Franck-Condon and Herzberg-Teller terms were included in the transition dipole and quadrupole moments. Right now, the experimental spectra are reproduced *ab-initio* only in a qualitative manner. Inclusion of all the non-resonating states might be necessary to get a reasonable correspondence between theory and experiment.

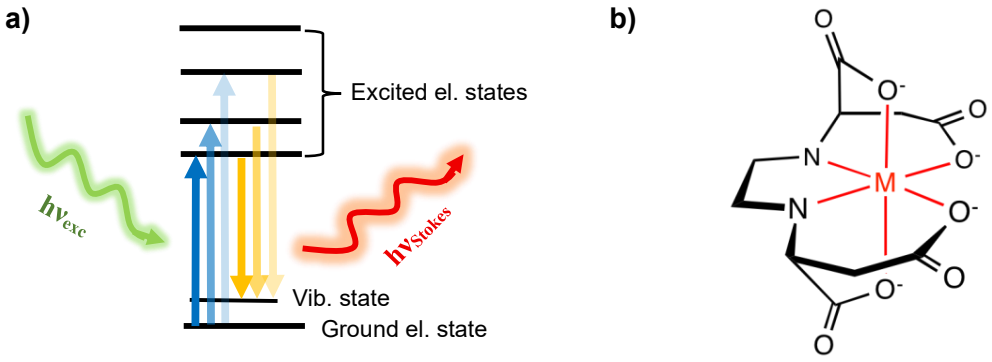


Figure 1. a) Jablonski diagram of resonant Raman scattering, the exciting light $h\nu_{exc}$ resonates with low-lying excited states and is then scattered with lower energy as $h\nu_{Stokes}$. **b)** The studied EDDS transition metal complex where ***M*** represents the transition metal atom (Co^{III}, Cr^{III}).

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Electrochemical Biosensor for Detection of Nucleic Acids Based on Biamperometric Approach

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This project focuses on the development of an electrochemical biosensor for detecting specific nucleic acid sequences. An electrochemical DNA biosensor provides a convenient alternative to conventional diagnostic methods, offering great potential in the field of point-of-care testing (POCT) due to its simplicity, low cost, and portability.[1] The concept relies on immobilizing DNA onto the surface of measuring electrodes, enabling monitoring of the electrochemical response in the presence or absence of single-strand DNA or double-strand hybridized complementary DNA. Electrochemical impedance spectroscopy (EIS) was used to characterize changes in the electrode surface. The detection principle was based on changes in charge transfer resistance (R_{ct}), which is influenced by the properties of the modification layer controlling the access of the redox probe to the electrode.[2] Several electrode modification strategies were evaluated. The most effective treatment was with poly-L-lysine, followed by DNA immobilization at room temperature. Electrochemical measurements were carried out at 60°C in a temperature-controlled ferro/ferricyanide solution to improve signal resolution. The hybridization event was successfully detected as significant changes in the electrochemical signal. These results were supported by complementary characterization methods: atomic force microscopy (AFM) enabled visualization of the electrode surface, while quantitative PCR (qPCR) independently verified sequence hybridization. Overall, this work demonstrates the potential of electrochemical biosensors for nucleic acid detection. The combination of optimized electrochemistry, sensitive impedance measurements, and independent validation highlights their applicability for future possible point-of-care diagnostics and related biomedical applications.

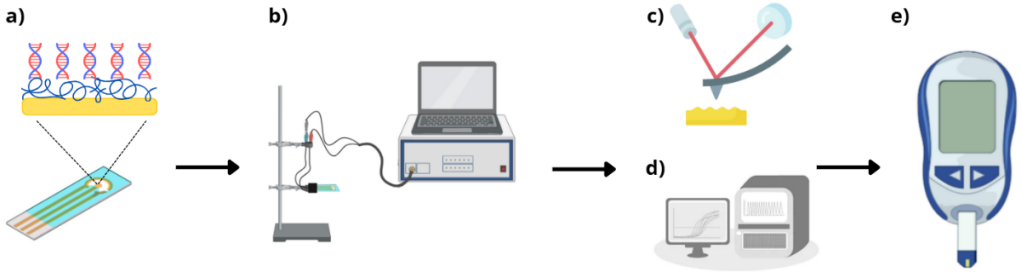


Figure 1. Development of an electrochemical DNA biosensor. Electrode modification with poly-L-lysine and immobilization of DNA (a). Detection of DNA by EIS (R_{ct}) (b). Validation by AFM and qPCR (c,d). Future possible point-of-care diagnostics device (e).

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