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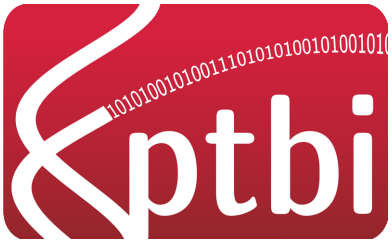

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Contents

Session VII

Lectures

Powering Drug Discovery with Structural Insight

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Structural biology, a cornerstone of drug discovery, offers profound insights into the three-dimensional structures of biological macromolecules, including proteins, nucleic acids, and viruses. Structural biology examines the integration of data acquired from diverse biochemical and biophysical techniques with those derived from structural techniques. Thus, structural information has been critical to drug discovery and structural bioinformatics. The integration of artificial intelligence (AI) into X-ray crystallography has shown great promise in automating and accelerating the analysis of complex structural data, further improving the efficiency and accuracy of structure determination.

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Genomics for Poland (G4PL): building a national genomic infrastructure for precision medicine and European integration

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Recent decades have witnessed a rapid expansion of national human genome initiatives worldwide, fundamentally transforming biomedical research, healthcare, and precision medicine. Large-scale genomic projects such as the UK 100,000 Genomes Project, the All of Us Research Program in the United States, and multiple European population sequencing efforts have enabled the systematic identification of disease-associated variants, development of personalized diagnostics, and implementation of genomics-driven healthcare. Despite these global advances, Poland has so far lacked a coordinated, nationwide human genomics initiative, resulting in fragmented datasets, limited standardization, and underrepresentation of the Polish population in international genomic resources.

To address this gap, the Genomics for Poland (G4PL) project was launched as a nationwide consortium integrating leading research institutes, universities, healthcare institutions, and computational infrastructure providers. The project aims to establish a national network of accredited genomic laboratories, develop advanced sequencing and bioinformatics infrastructure, and create a centralized genomic repository enabling harmonized analysis and secure data sharing. Particular emphasis is placed on long-read sequencing technologies, re-analysis of existing datasets, development of standardized analytical pipelines, and implementation of artificial intelligence methods for genomic interpretation.

A key objective of G4PL is to generate new genomic collections, including data from patients with rare diseases and representatives of the Polish population, thereby creating the Polish contribution to the Genome of Europe initiative and facilitating Poland's active participation in European genomic programs. By establishing sustainable infrastructure and interoperable standards, G4PL seeks not only to strengthen national genomic capacity but also to enable Poland to catch up with leading countries in human genomics and become an active contributor to the European genomics ecosystem.

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Combining AI and experiment

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Deep-learning structure predictors such as AlphaFold have transformed structural biology, yet they still struggle with several challenging cases: antibody-antigen complexes, host-pathogen interactions, alternative conformations, and very large assemblies. In this talk I will first examine how AlphaFold performs on modeling such interactions I will then introduce AF3x, our extension of AlphaFold 3 that incorporates crosslinking mass spectrometry as explicit ligand-like restraints and substantially improves models of antibody-antigen, host-pathogen, and multi-conformation systems. Finally, I will show how combining AF3x with in-cell crosslinking mass spectrometry opens a route to mapping protein-protein interactions in their native cellular context, illustrated by our ongoing work on influenza virus-human interactions.

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Predicting Locations of Cryptic Pockets from Single Protein Structures Using the PocketMiner Graph Neural Network

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Cryptic pockets expand the scope of drug discovery by enabling targeting of proteins currently considered undruggable because they lack pockets in their ground state structures. However, identifying cryptic pockets is labor-intensive and slow. The ability to accurately and rapidly predict if and where cryptic pockets are likely to form from a structure would greatly accelerate the search for druggable pockets. Here, we present PocketMiner, a graph neural network trained to predict where pockets are likely to open in molecular dynamics simulations. Applying PocketMiner to single structures from a newly curated dataset of 39 experimentally confirmed cryptic pockets demonstrates that it accurately identifies cryptic pockets (ROC-AUC: 0.87) >1,000-fold faster than existing methods. We apply PocketMiner across the human proteome and show that predicted pockets open in simulations, suggesting that over half of proteins thought to lack pockets based on available structures likely contain cryptic pockets, vastly expanding the potentially druggable proteome.

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From scores to insight: visual analytics for RNA structure prediction

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The recent rise of machine learning has transformed the landscape of RNA 3D structure prediction, accelerating method development and attracting new researchers to the field. Despite these advances, assessing model quality remains a major challenge. Global evaluation metrics such as RMSD, TM-score, and INF provide useful summaries of prediction accuracy but often fail to explain the underlying causes of modeling errors. In this talk, I will demonstrate how visual analytics can support the assessment of RNA 3D structures by combining global quality measures with local, structure-aware analyses. Using examples from RNA-Puzzles and CASP experiments, I will show how geometric, steric, and topological analyses reveal regions of correct and incorrect prediction that remain hidden behind global scores. These examples illustrate how multi-scale visualization can facilitate error diagnosis, improve model interpretation, and provide insights into the strengths and limitations of current RNA structure prediction methods.

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Advances and Bottlenecks in RNA 3D Structure Modeling

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Accurate RNA structure prediction is critical for understanding RNA function and supporting RNA-targeted therapeutic design. Here, we evaluate current RNA 3D structure prediction methods through comparative benchmarking studies and community-wide blind experiments, including our participation in RNA-Puzzles, CASP15, and CASP16, with the recent RNA-Puzzles community assessment described in (1). Our analyses reveal complementary strengths between machine learning (ML) and physics-based approaches. ML methods perform well in recovering global RNA fold topology, whereas physics-based methods more effectively capture local interactions and fine structural details (2). We further show that molecular dynamics refinement functions primarily as a local optimization strategy and remains strongly dependent on the quality of the starting model. Results from CASP16 highlight both substantial progress and persistent challenges in the field (3). Notably, our group achieved first place in the RNA multimer category. At the same time, our structural analysis shows that multi-helix junctions as major sources of error propagation, particularly in RNA multimer prediction (3).

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OpenRNAFold: an end-to-end approach for RNA 3D structure prediction

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RNA plays central roles in gene expression as messenger RNA and in many biological processes as structured non-coding RNAs. Understanding these functions requires knowledge of RNA tertiary structure, but experimental structure determination is slow and expensive, leading to a large gap between sequence and structure data.

Inspired by advances in protein structure prediction such as AlphaFold, RNA structure prediction has attracted growing interest. However, current deep learning models for RNA often report inflated accuracy due to structural overlap between training and test sets. Early benchmarks also indicate that AlphaFold3 remains inaccurate for RNA structures.

Because RNA structures are scarce, we focus on two essentials: carefully curated training data to prevent data leakage (RNA3DB, Szikszai, et al., 2024), and high-quality sequence alignments (http://rnahub.org, Magnus, et al., 2025). Using these datasets and alignments, we are currently testing our own AlphaFold-based RNA model that explicitly emphasizes base pairing and RNA geometry.

This work aims to improve RNA 3D structure prediction and is being evaluated in upcoming blind challenges such as CASP and RNA-Puzzles. It will also serve as a platform for further deep learning developments in RNA design and therapeutics in my Laboratory of RNA Design and Therapeutics at CeNT, University of Warsaw.

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RNA Recognition Motif, but Not for RNA: The GW182 SD RRM Controls Liquid-liquid Phase Separation and CNOT1 Recognition

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GW182 is a key fuzzy, protein that binds the CNOT1 subunit of the 3' deadenylase CCR4- NOT complex, thereby driving post-transcriptional microRNA-mediated gene silencing (1). The C-terminal silencing domain (SD) of GW182 is almost completely intrinsically disordered (2), except for the conserved RNA Recognition Motif (RRM). While the crucial GW182 sequence motifs responsible for interactions with CNOT1 have been characterized (3), the putative role of the RRM has remained elusive (4).

Here, we integrate in silico conformational modelling, fluorescence correlation spectroscopy (FCS), and liquid-liquid phase separation (LLPS) to unravel the role of the RRM in the GW182 SD protein. The molecular modelling results suggest that the RRM maintains the SD in a more open and dynamic conformational ensemble, whereas RRM deletion promotes compaction of the protein, increasing intramolecular contacts and reducing the accessibility to tryptophan residues. Experimentally, using FCS and LLPS, we find that the SD undergoes LCST-type LLPS through a tryptophan-dependent mechanism only when the RRM is present. Its absence strongly impairs efficient condensate formation, yields less solvent-accessible ensembles, and alters interactions with CNOT1. Together, these results support a model in which the RRM functions as a conformational gate that maintains short linear motifs (SLiMs) and tryptophan residues in separated, solvent-accessible states that enable LLPS and CNOT1 recognition. By contrast, RRM deletion promotes compaction of these interaction-prone regions into less accessible conformations, thereby preventing efficient condensate formation and reducing interactions with CNOT1.

This work was supported partially by the NCN grants; no. UMO-2016/22/E/NZ1/00656 to A. N, and no. UMO-2023/49/B/NZ1/04320 to M. K. B. The studies were performed in the NanoFun laboratories co-financed by ERDF within the POIG.02.02.00-00-025/09 project.

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Machine learning guided eccDNA prediction based on gene locus data, as a PU learning problem

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Introduction Extrachromosomal circular DNA (eccDNA) constitutes a heterogeneous class of covalently closed, circular DNA molecules, which may be either double- or single-stranded and range in size from tens of base pairs to several megabases (approximately 100 bp to 5 Mbp). Most proposed mechanisms of eccDNA biogenesis implicate chromosomal microdeletions or DNA breakage events, resulting in the excision, looping, and subsequent circularisation of DNA fragments. Notwithstanding these proposals, the precise mechanisms underlying eccDNA formation remain incompletely characterised. Furthermore, there is a paucity of empirical evidence concerning the factors that modulate the likelihood of a given genomic sequence being represented as eccDNA.

Recent advances in artificial intelligence and computational biology, coupled with growing interest in eccDNA, have facilitated the development of initial predictive models. These approaches are predominantly sequence-based, rendering them computationally demanding and constraining both their interpretability and their capacity to yield broader biological insight.

Methods Drawing upon the existing literature and publicly available datasets, we identified a set of candidate features, derived principally from genomic locus and contextual information, that may contribute to eccDNA formation. Statistical analyses were conducted on a curated, randomly sampled set of sequences to assess the relevance of these features. Building on this, we employed a positive-unlabelled (PU) learning framework, an approach not yet widely adopted in this domain, to develop a predictive model without recourse to explicitly labelled negative samples. To optimise model performance, a range of contemporary machine learning algorithms was evaluated and their effectiveness for this task systematically compared.

Results The statistical analyses revealed previously unreported associations between selected genomic features and known eccDNA regions. In addition, pairwise analyses identified non-trivial relationships among locus-derived features. The resulting model permits eccDNA prediction without reliance on sequence data and demonstrates strong performance according to PU learning evaluation metrics reported in the literature.

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R3BIND: Decoding protein-nucleic acid recognition through 3D structural templates

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Protein-nucleic acid (protein-NA) interactions are central to gene regulation and genome maintenance, underpinning processes such as transcription, splicing, DNA repair, and viral replication. Their dysregulation is associated with numerous human diseases, including cancer, neurodegeneration, and infection, highlighting the importance of understanding these interactions at the structural level. Although structural databases of protein-NA complexes have expanded rapidly, there remains no systematic framework for identifying, comparing, and predicting protein-binding interfaces across RNA- and DNA-containing structures. This limitation hinders the transfer of structural knowledge between related complexes and restricts the discovery of conserved interaction principles. To address this gap, we are developing a computational framework for the automated identification and annotation of protein-NA interfaces using three-dimensional structural information that is not captured by sequence-based approaches alone. The approach combines a curated library of high-quality protein-NA binding interfaces with the in-house developed ARTEM structural superposition algorithm, enabling large-scale detection of recurring interaction motifs across structural databases. This platform is designed to reveal conserved structural determinants of nucleic acid recognition and to support systematic comparison of interfaces across diverse protein-NA assemblies. The resulting resource will facilitate functional annotation of newly characterized structures, provide mechanistic insight into protein recognition of nucleic acids, and support the identification of structurally conserved interaction hotspots with potential therapeutic relevance. More broadly, this work establishes a scalable strategy for connecting structural patterns to biological function across the protein-NA complexes.

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Computational Design and Optimization of Blood-Brain Barrier Modulating Peptides

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The delivery of pharmaceutical agents to the central nervous system is hindered by the Blood-Brain Barrier (BBB). We present an extensive computational search for novel peptides able to modulate the BBB, focusing on cadherin proteins, which are one of the components of intercellular junctions forming the BBB. Starting with two known active sequences, HAV and ADT, we discovered $\tilde{1}$00 cadherin-binding peptides derived from E-cadherin using classical docking. Further computational search for BBB modulators was accelerated with Artificial Intelligence (AI) approaches. This involved using AlphaFold3 for generating cadherin-peptide complexes, estimation of binding free energies with PRODIGY and exploration of sequence space through mutations. Additionally, diffusion-based AI approaches were employed to expand sequence search. Overall, $\tilde{3}$,000 sequences were explored, leading to prediction of several sequences with very high cadherin affinity, as well as sequences with specific preference for two isoforms, E- and VE-cadherin. Biological activity and pharmacological properties of these systems still require experimental validation. Overall, this work presents a systematic computational approach for generating novel peptides with high potential for disrupting the BBB and contributing to the treatment of diseases of the central nervous system, such as Alzheimer's and other neurodegenerative illnesses.

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A novel, widespread class of protein misfolding is associated with aging and disease

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Protein misfolding is typically associated with aggregation, amyloid formation, or global unfolding. Here, I will describe a new, distinct class of soluble, near-native misfolding involving non-covalent lasso entanglements, in which proteins remain compact but adopt altered topologies that can impair function and challenge protein homeostasis in cells. Using structural annotations, proteome-scale limited proteolysis mass spectrometry, disease databases, pathogenic mutation datasets, and molecular simulations, we find that native entanglement misfolding is associated with both aging and disease. In aging Saccharomyces cerevisiae, entanglement-prone proteins are more likely to undergo age-associated structural changes, particularly within natively entangled regions. In the human proteome, natively entangled proteinsare enriched for disease association, pathogenic missense mutations, and pathogenic mutations in misfolding-prone regions. Together, these results support a novel model in which native entanglements create topological vulnerabilities in folded proteins that, with age or mutation, promote soluble nonfunctional misfolded states. This mechanism defines a new widespread classof proteostasis failure and suggests new opportunities to prevent topological misfolding relevant to aging and disease.

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Comparative molecular-dynamics study of dibenzoylmethane and emodepside in BK potassium channels (6V38, 7PXH)

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Large-conductance Ca(2+)-activated potassium (BK) channels are key therapeutic targets in both mammalian (KCNMA1 channelopathies) and parasitic (nematode SLO-1) systems. We previously identified DIBENZOYLMETHANE (DBM), the bioactive β-diketone of Hottonia palustris extracts, as an occupancy-dependent pore blocker of human BK: inside-out patch-clamp on U87 MG cells showed reduced open probability, and AutoDock + MD on the human BK pore (PDB 6V38) confirmed that four DBM molecules near the cytosolic exit of the selectivity filter fully abolish K(+) flux, while one or two ligands give a partial block, both accompanied by loss of vestibular water. These DBM results are now available as a bioRxiv preprint (Koprowski et al., DOI: 10.64898/2026.04.24.720584).

Here we extend the pipeline to EMODEPSIDE, the semi-synthetic cyclooctadepsipeptide anthelmintic. We performed all-atom MD (NAMD, CHARMM/CGenFF) on five complementary systems with and without a transmembrane electric field: (i) apo 6V38, (ii) 6V38 + DBM, (iii) 6V38 + emodepside, (iv) apo 7PXH, and (v) 7PXH + emodepside. System (v) uses the cryo-EM structure of Drosophila BK/Slowpoke (PDB 7PXH); in (iii) emodepside was placed in human BK by structural analogy with 7PXH, not by docking. Dedicated CHARMM dihedral parameters for the cyclooctadepsipeptide backbone and morpholine rings were derived and validated by QM.

Across all five trajectories, the apo channels show stable K(+) permeation, while every ligand-bound system displays reduced flux and decreased inner-vestibule hydration. Emodepside, despite being two orders of magnitude larger than DBM, modulates K(+) flow through the same geometric mechanism: physical occlusion of the cytosolic exit of the selectivity filter combined with disruption of the structured water network. The transmembrane field amplifies these effects and supports a unified, voltage-sensitive mechanism of BK-channel block transferable between human (6V38) and Drosophila (7PXH) orthologues.

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Comptational Modeling of Proteins Involved in Regulating Cell Fate

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Recent years have brought attention to ferroptosis, an iron- and lipid peroxidation-dependent form of regulated cell death implicated in a broad range of diseases, including Alzheimer's and Parkinson's disease, acute brain injury, sepsis, or asthma. Ferroptosis's characteristic feature is enhanced lipid peroxidation, where abstraction of H-atoms from polyunsaturated phospholipids drives the entire peroxidation process, causing membrane damage and leading to cell death.

Our primary objective is to target the enzymatic mechanisms underlying ferroptosis at the molecular level. By employing computational approaches such as molecular dynamics simulations, molecular docking, elastic network models, and bioinformatics, in conjunction with the experimental verification, we elucidated previously unknown mechanisms and factors affecting or blocking ferroptosis, thus providing molecular insights of the catalytic processes involved (Cell2017, JACS 2018, J Clin Invest 2018, JCIM 2019, Free Redox Biol Med 2023, Angew Chemie Int Ed 2024, Redox Biol 2025, Nature Commun 2026).

Furthermore, our recent studies uncover the critical role of iNOS/nitric oxide (Nature Chem Biol 2020, IJMS 2021) and phospholipase iPLA2β (Nature Chem Biol 2021) in regulating ferroptosis. Additionally, we have resolved a paradox concerning Ferrostatin-1, the most commonly used ferroptosis inhibitor (Redox Biol 2021), and we proposed new inhibitors of the human complex that can effectively block the ferroptotic cell death signal (PNAS 2023). Through our research, we have demonstrated the efficacy of computational modeling methods in elucidating and inhibiting fundamental biological processes.

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Exploring the genomic landscape of Neolithic human populations

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Ancient DNA provides an opportunity to investigate the patterns of genetic diversity and disease-associated variation in past human populations. This study aimed to explore genetic variation among Neolithic agrarians, hunter-gatherers, and Late Neolithic populations, focusing on differences in the distribution of potentially pathogenic genetic variants among these groups.

Whole-genome sequencing data from agrarians (n = 13), hunter-gatherers (n = 8), and Late Neolithic individuals (n = 10) were processed using the EAGER ancient DNA pipeline with custom read trimming and merging procedures prior to alignment against the reference genome and SNP calling. Variants were filtered using quality control procedures, and samples with excessive missing data were excluded. The population structure was assessed using multidimensional scaling (MDS) based on Manhattan distances. Functional analysis was performed by aggregating deleterious variants (CADD > 10) into gene-level burden scores, followed by Gene Set Variation Analysis (GSVA) and statistical testing of pathway activity differences between populations. Polygenic risk scores (PRS) for inflammatory bowel disease (IBD), type 2 diabetes (T2D), and body mass index (BMI) were calculated and compared between populations.

MDS revealed a clear clustering of individuals according to their population groups. GSVA identified multiple significantly enriched biological processes, molecular functions, and pathways (GO, Hallmark, KEGG, and Reactome), revealing distinct functional profiles among the three populations. Comparative analyses revealed population-specific patterns in the distribution of potentially pathogenic variants. Significant differences in PRS were observed among the three populations for all investigated traits: BMI, IBD, and T2D.

The study provides insights into the genomic landscape of ancient European populations and highlights the differences in the distribution of potentially pathogenic variants among Neolithic agrarians, hunter-gatherers, and Late Neolithic groups. While the results do not directly indicate disease mechanisms, they contribute to a better understanding of the evolutionary history of clinically relevant genetic variation and population-specific genetic diversity in prehistoric Europe. This project was funded by the National Science Centre (NCN), Poland, under grant no. 2017/26/E/NZ5/00851. High-performance computing resources were provided by the Poznan Supercomputing and Networking Center (PCSS).

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Longitudinal analysis of sinonasal microbiome in chronic rhinosinusitis

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Most studies on the sinonasal microbiome were conducted at a single time point and limited to genus-level resolution, leaving the temporal dynamics of the microbial community in this niche poorly understood. Chronic rhinosinusitis (CRS) is among the most prevalent chronic inflammatory diseases of the upper airways, yet the role of the microbiome in disease progression and acute exacerbations remains unclear.

We collected longitudinal sinonasal samples from CRS patients at a minimum of four time points, alongside samples from healthy controls. Full-length 16S rRNA gene sequencing was performed using Oxford Nanopore Technologies, enabling species-level taxonomic resolution. Sequencing-based profiles were compared with results from routine hospital microbiological cultures.

The sinonasal microbiome exhibited high inter-individual variability in both CRS patients and healthy controls, with no consistent taxonomic signatures distinguishing the two groups. Within the heterogeneous CRS cohort, shifts in microbiome composition and temporal dynamics coincided with episodes of symptom worsening in a subset of individuals. Building on these observations, we developed an approach to identify microbial blooms associated with bacterial exacerbations.

Our work demonstrates how advanced sequencing technologies can enhance clinical diagnostics and bring us a step closer to precision medicine.

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From genes to lifestyle changes: multiomics analysis of pigs' liver transcriptome in context to modern lifestyle diseases

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The presentation will explore an international research project based on the in vivo and in vitro experiments of hepatoprotective action of medicinal herbs in pigs as an animal model. The presentation includes the multilevel molecular analysis covering transcriptomic, proteomic, and metabolomics studies on pig liver, and comparison of gene expression profiling between pig vs human hepatocytes in the context of modern lifestyle diseases.

Acknowledgement This research is financed and supported by the international scientific project NCN-OPUSLAP (UMO-2021/43/I/NZ9/02612) entitled: Multilevel molecular analysis of the hepatoprotective effect of medicinal herbs extracts in prevention of liver dysfunction caused by aflatoxin B1 in pig as an animal model (in-vivo), and hepatocyte cell culture analysis in human and pig (in-vitro).

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Applications of Machine Learning Methods to Datasets with a Small Number of Samples

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Post-transplant lymphoproliferative disorder (PTLD) is a rare but serious complication of organ transplantation. Machine learning analysis of PTLD is particularly challenging because available datasets contain very few samples, originate from different studies, and involve tens of thousands of molecular variables. In this work, we investigated whether robust machine learning techniques can extract meaningful biological information and provide reliable classification despite these limitations.

Gene expression profiles from 45 subjects were analyzed to distinguish three classes: 23 EBV-positive PTLD patients, 10 EBV-negative PTLD patients, and 12 non-PTLD kidney transplant recipients. Relevant biomarkers were identified using a combination of all-relevant feature selection methods, including Multidimensional Feature Selection (MDFS), Boruta, and Robust Aggregative Feature Selection (RAFS). The final panel consisted of 18 genes associated with antiviral response, immune regulation, and cellular stress.

To reduce overfitting, we developed an ensemble of weighted Naïve Bayes classifiers built from all combinations of selected features. Individual feature contributions were adjusted according to inter-feature correlations, and classifier outputs were aggregated across the ensemble. Model performance was evaluated using repeated stratified Monte Carlo cross-validation involving 2000 independent train-test splits. Despite the extremely small sample size, the proposed approach achieved highly accurate and stable classification. Across all validation runs, only a single EBV-positive sample was consistently misclassified. The results demonstrate that ensembles of simple probabilistic classifiers can provide robust predictive performance in small-sample, high-dimensional biomedical datasets while retaining biological interpretability. The identified biomarkers also revealed meaningful molecular differences between PTLD subtypes and may have potential diagnostic value.

Harnessing Artificial Intelligence

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Development of multi-agent systems that support scientific research has already led to important discoveries in different branches of science. Physics-informed machine learning systems trained to respect constraints based on the natural laws are successfully applied to analyze complex real-world phenomena, from quantum systems, molecular biology to weather prediction. AI algorithms are used to design new experiments, making discoveries at the conceptual level. Self-improving models can significantly increase their performance. A new paradigm for creating software and solving problems has emerged: interpreting commands, developing skills, using tools, and orchestrating the actions of multiple agents.To address grand challenges new research scientist agent platforms are being created, involving humans supported by teams of specialized AI agents with specific types of intelligence adjusted to the particular needs and domains of research. To stay at the front of scientific research we have to build such platforms and learn how to use them. A new era of AI4Science is coming, featuring OpenClaw-style agents capable of autonomous execution of numerous scientific projects.

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Dynamics and mechanism of photoreactions in flavo-enzymes

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In exceptional cases, flavo-enzymes perform functional light-driven catalysis, such as in fatty acid photodecarboxylase (FAP)[1-2]. Yet most display light-independent functions, although in these photophysical processes also occur. Such processes can have photoprotective functions, and may also be exploited for photocatalysis or photoswitching applications [3]. Here, recent ultrafast spectroscopic studies on FAP and on short-lived photoproducts in "nonphotoactive" flavoproteins exploring various redox and ligation states will be surveyed. They include the discovery of two hitherto unknown photoreactions that occur on the timescale of a few hundred femtoseconds or less. First, we observed quasi-instantaneous (<100 fs) photo-oxidation of anionic flavin radicals in various flavoprotein oxidases, and subsequent charge re-separation in a few tens of picoseconds [4]. Such a non-functional photoreaction also occurs in FAP, where it surprisingly involves hydrated electron intermediates [5]. Second, we studied the charge-transfer complex formed by the flavin ring system and the substrate-analog inhibitor methylthioacetate in monomeric sarcosine oxidase [6]. Here, upon population of the photo-excited CT state, with near-unity quantum yield a state spectroscopically identical to the non-complexed enzyme is formed in 300 fs in a barrierless process. This implies that all CT interactions are vanished on this timescale. The initial CT complex is subsequently recovered in a strongly thermally activated way on the nanosecond timescale. These are properties of a highly efficient red-absorbing photoswitch. The possible ultrafast structural changes associated with this unprecedented process are discussed, as well as new in-depth characterizations of the process, and possible extensions of this system for practical applications [7].

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CABS-flex 3.0: Web Server and Standalone Workflows for Protein Flexibility and Peptide Modeling

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Protein flexibility is essential for molecular recognition, regulation, catalysis, and protein engineering. CABS-flex is a computational method for fast simulations of protein and peptide conformational dynamics using the CABS coarse-grained model followed by all-atom reconstruction. The method has been developed through several generations and is currently available as the CABS-flex 3.0 web server, which provides an accessible interface for protein flexibility simulations, linear and cyclic peptide modeling, ensemble visualization, and convenient analysis of simulation results (1). In this talk, I will present the current CABS-flex ecosystem, focusing on the CABS-flex 3.0 web server and the upcoming CABS-flex standalone 3 package. Recent developments include improved restraint-generation schemes, AlphaFold pLDDT-guided flexibility modeling, peptide modeling workflows, and updated all-atom reconstruction tools (2,3). The standalone version extends these capabilities into a reproducible command-line platform for customizable protein flexibility simulations, peptide modeling, and flexible peptide–protein docking. Together, the web server and standalone package provide complementary access to CABS-based modeling: an intuitive online interface for broad use and a flexible command-line platform for advanced and reproducible simulations.

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Unmasking the Data: Dedicated Methods for LCR Analysis and Annotation Transfer

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For decades, Low-Complexity Regions (LCRs) in proteins were dismissed as evolutionary "noise" and routinely masked in standard bioinformatic pipelines. Today, they are recognized as important functional elements, driving critical biological processes such as Liquid-Liquid Phase Separation (LLPS) and playing key roles in neurodegenerative diseases. However, a fundamental methodological problem persists: the scientific community frequently attempts to analyze LCRs using tools explicitly designed and optimized for high complexity sequences and structured, globular proteins. This presentation examines the critical limitations and pitfalls of applying standard algorithms to analysis low complexity sequences. We will discuss how classical approaches for protein sequence similarity comparison such as BLAST fail when applied to LCRs. To overcome these algorithmic blind spots, a new set of tools and methods for LCR analysis is required. Implementing these dedicated algorithms is essential for the accurate transfer of functional annotations, unlocking a deeper understanding of how these regions drive biological processes.

From Sequence to a Knot - using Large Language Models to Predict the Existence of a Knot in a Protein Directly from its Sequence

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Knotted proteins are rare but strictly conserved within certain families, yet why they form has remained unclear due to limited data. Using AlphaFold's predictions for the entire UniProt database (∼200 million proteins), we identified ∼700,000 knotted proteins (under 0.35%) falling into just 15 superfamilies, and trained a machine learning model (fine-tuned ProtBert-BFD) that predicts knotting from sequence alone with 98.5% accuracy.

Although every family appeared to contain both knotted and unknotted members, detailed analysis showed that in most families the "unknotted" proteins are just nonfunctional fragments missing part of the knot core – so the knot is genuinely conserved in functional proteins.

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PROTO-NOOS: Orchestrating Open-Access Bioinformatics for Seamless Drug Discovery

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Computational antibiotic discovery requires a reproducible workflow that links chemical generation, structural modelling, biological interpretation, and synthetic feasibility. This study presents **PROTO-NOOS**, a modular *in silico* pipeline for prioritising small-molecule candidates against *Escherichia coli* dihydrofolate reductase (DHFR). The workflow combines *de novo* molecule generation, physicochemical and Gram-negative entry filtering, affinity prediction, molecular-dynamics-based stability assessment, metabolic and target-level interpretation, and retrosynthetic accessibility analysis.

A mechanistic **ODE-based Cell Target Engagement** layer extends affinity-based ranking by modelling compound entry, efflux, and target binding inside the bacterial cell. This allows candidates to be evaluated not only by their predicted binding strength, but also by their expected ability to reach and engage the intracellular target.

Rather than relying on a single affinity score, the pipeline ranks compounds using multiple complementary signals. Consequently, the highest-priority candidates are not interpreted as confirmed antibacterial hits, but as systems selected for deeper mechanistic follow-up. Selected protein–ligand–cofactor complexes can be transferred to a separate downstream workflow focused on binding-site stability, interaction patterns, and the physical plausibility of predicted binding modes, with future extensions including enhanced sampling and QM/DFT inspection where needed.

The current study establishes this workflow as an *in silico* prioritisation framework. Its outputs—including short-MD stability proxies and systems-biology labels—are best understood as **ranking signals** that identify candidates worth testing further. Experimental synthesis, antibacterial assays and wet-lab validation form the planned next step, where these computational priorities can be evaluated against measurable biological activity.

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Epitope-driven molecular docking and structural characterisation of B10 camelid antibody against SARS-CoV-2 virus

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The SARS-CoV-2 virus relies on its interactions with the host receptor ACE2 in order to infect cells. Binding occurs between the Receptor-Binding Domain (RBD) of the SARS-CoV-2 spike protein and the extracellular domain of the host's membrane-embedded ACE2 receptor. Among the diverse strategies to combat this virus, camelid-derived heavy-chain-only antibodies (hcAbs) and their variable VHH domains have emerged as highly potent therapeutic candidates. The main advantages are their small size, stability, and good tissue penetration. In this study [1], the novel camelid antibody B10 was discovered using a naive phage display library and extensively characterized experimentally. While in vitro ELISA and neutralization tests demonstrated that B10 possesses a strong neutralizing capacity against the original Wild-type (WT) and Omicron variants, the Delta variant was shown to undergo complete immune evasion.

To decipher the structural features and molecular mechanisms underlying B10's neutralizing capability, we performed a targeted molecular modeling study. Experimental epitope mapping data highlighted two critical interaction regions within the RBD domain (L441–D467 and Y489–V511). These fragments were directly utilized as data-driven distance restraints to drive molecular docking simulations in HADDOCK v2.4.

Our computational docking results revealed a strong structural and energetic preference for the B10 antibody to bind the SARS-CoV-2 Spike trimer in its "down" (closed) conformation across the sensitive variants. Refined models of the full-length heavy-chain antibody (hcAb-B10) demonstrated stable, bivalent engagement with two closed RBDs simultaneously, yielding highly favorable free binding energies. This interaction effectively locks the Spike trimer in its closed state, physically and sterically preventing the conformational transition to the "up" state required for ACE2 receptor engagement. Conversely, docking simulations with the Delta variant successfully captured the structural basis for immune evasion, where the hallmark L452R mutation introduces a conformational shift that buries the antibody's target loop, resulting in unstable and shifted poses. This epitope-driven molecular modeling study provides a clear molecular explanation for the mechanism of action of the B10 antibody against SARS-CoV-2. By complementing and structurally rationalizing the experimental data, our findings highlight the power of targeted molecular modeling in evaluating, understanding, and optimizing therapeutic candidates against mutating viral pathogens.

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An Automated Pipeline Combining Hidden Markov Models, Structural Alignment, and Keyword Mining for Systematic Identification and Classification of Cytochrome P450s in the Protein Data Bank

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Cytochrome P450 monooxygenases (CYPs/P450s) form a highly diverse enzyme superfamily central to biotechnology, pharmacology, and environmental science. Despite the large number of available structures, identifying and comparing P450 entries in structural repositories remains challenging due to their extreme sequence divergence and inconsistent annotation practices. In particular, many deposits lack the standardized nomenclature (*CYPid*) and rather rely on legacy or author-defined *common names* (like *P450cam*, *P450BM-3* and *P450-PCN1*), which are often inconsistent in formatting and specificity. This is particularly difficult for a superfamily as sequentially diverse as P450s. This hinders reliable retrieval and cross-referencing, making even identification all P450 structures in the database nontrivial. To overcome these obstacles, we developed a structure-guided discovery and validation workflow combining keyword search, Hidden Markov Models, and structural alignment, enabling robust detection and annotation. This strategy identified 1,513 deposits representing 674 unique sequences. All sequences were reannotated using the P450Atlas server and manually verified, confirming high assignment accuracy. In the process, we have also identified five new CYP subfamilies. The resulting dataset constitutes the first rigorously curated, structure-linked registry of P450 enzymes, integrated into a publicly accessible resource and supported by an automated pipeline that periodically scans newly released entries. By unifying structurally validated identification with standardized CYP nomenclature, this work establishes a reliable framework for accurate retrieval, comparison, and future large-scale analyses of P450 enzymes.

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Molecular dynamics simulations reveal dimerization-dependent rearrangement of the tyrosine toggle switch in the histamine H4 receptor

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The histamine H4 receptor (H4R) is a G protein-coupled receptor involved in immune and inflammatory signaling and is considered a promising therapeutic target in allergic, inflammatory, and immune-mediated disorders, including atopic dermatitis, pruritus, asthma, allergic rhinitis, chronic arthritis, and inflammatory bowel diseases. H4R may exist both as a monomer and as a functional homodimer, but the structural and dynamic consequences of receptor dimerization remain incompletely understood. In this study, we investigated the differences between the monomeric and dimeric forms of H4R using molecular dynamics simulations. A total of 56 independent MD simulations were performed: 20 simulations of the H4R monomer and 36 simulations of the H4R–H4R homodimer, including agonist-bound, antagonist-bound, and apo states. Comparative analysis revealed a systematic difference between the monomeric and dimeric systems in the configuration of the tyrosine toggle switch associated with the NPxxY motif. In all 20 monomer simulations, Y7.53 formed a π–π stacking interaction with F2.43, whereas this interaction was absent in all 36 dimer simulations. The observed rearrangement occurs away from the dimer interface, suggesting that dimerization may propagate structural effects through the receptor core. Because the NPxxY-associated tyrosine switch is involved in GPCR activation, these results suggest that H4R dimerization may modulate receptor conformational dynamics and activation-related microswitches. This indicates a possible allosteric-like role of dimerization in regulating H4R activity and provides a structural hypothesis for further experimental and computational studies.

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Accelerating Drug Discovery and Repurposing by Combining Transcriptional Signature Connectivity with Docking and Large Language Models

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We present an in silico approach for drug discovery, dubbed connectivity enhanced structure activity relationship (ceSAR). Building on the landmark LINCS library of transcriptional signatures of drug-like molecules and gene knockdowns, ceSAR combines cheminformatic techniques with signature concordance analysis to connect small molecules and their targets and further assess their biophysical compatibility using molecular docking. Candidate compounds are first ranked in a target structure–independent manner, using chemical similarity to LINCS analogs that exhibit transcriptomic concordance with a target gene knockdown. Top candidates are subsequently rescored using targeted docking simulations and machine learning–based consensus of the two approaches. Using extensive benchmarking, we show that ceSAR greatly reduces false-positive rates, while cutting run times by multiple orders of magnitude and further democratizing drug discovery pipelines. We further demonstrate the utility of ceSAR by identifying and experimentally validating inhibitors of BCL2A1, an important antiapoptotic target in melanoma and preterm birth–associated inflammation.

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Posters

Non-canonical interactions in RNA 2D structures

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Non-canonical base pair interactions play an important role in RNA structure and function, yet their systematic characterization remains challenging. Here, we present a pipeline for analyzing RNA secondary structures enriched with information on non-canonical base pairs. The analysis is performed on high-quality reference sets of RNA structures derived from the Protein Data Bank. Using the RNAPolis workflow, outputs from multiple annotation tools, including BPNet, DSSR, FR3D, MAXIT, RNAView, and DNATCO, can be integrated into a unified JSON-based representation of RNA 2D structures. This representation enables the identification of structural motifs such as stems, hairpins, loops, and single-stranded regions. We then detect non-canonical interactions and classify them according to the structural elements connected by the interacting nucleotides, considering intraloop, interloop, loop–hairpin, loop–single-strand, and loop–stem interactions. The proposed approach supports statistical analyses of non-canonical interactions across the dataset, including their distribution across different loop topologies and interaction classes, i.e., the frequency, mean, and median number of non-canonical base pairs by loop type.

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Investigating the PEBP1/LC3 complex: its dynamics, allostery, mechanism, binding regions and the role it plays in cell death pathways

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PE-binding protein 1 (PEBP1 or RKIP) is a multitasking, versatile protein present in the human body that participates in or initiates various cell death programs, such as ferroptosis and autophagy [1]. When the course of the degradation pathway mechanism is incorrect, it can lead to the development of various illnesses, e.g., cancer, Alzheimer's, multiple sclerosis, and pneumonia; therefore, comprehension of PEBP1, its behavior, binding partners, and mechanisms activated or inhibited by it, is crucial to furthering the knowledge in drug design and therapies [2].

One of PEBP1's potential partners is microtubule-associated protein 1b light chain 3 (LC3), which is assumed to bind through the LIR-motif to LC3 [3] [4]. The formation of the PEBP1/LC3 complex negatively influences autophagy, but the underlying mechanism remains poorly understood [3]. Therefore, the study was conducted to obtain the spatial structure, interaction dynamics, and binding regions of the LC3/PEBP1 complex using molecular dynamics (MD) simulations and molecular docking. Understanding this mechanism is important because autophagy plays a key role in maintaining cellular homeostasis in the human body. Clarifying how this interaction works is essential for further research into the potential activation or inhibition of this pathway and, consequently, for broadening our understanding of the functional versatility of the PEBP1 protein. The results indicate the successful determination of the complex's stable spatial structure and potential binding motifs.

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Machine learning-based graph analysis of cardiac intercellular communication networks in autoimmune myocarditis

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Experimental autoimmune myocarditis (EAM) induces extensive remodelling of cardiac intercellular communication networks. However, whether therapeutic intervention rewires these networks or primarily modulates existing signalling architecture remains unclear. Here, we applied graph-theoretic machine learning to determine whether disease state and metformin treatment are encoded within the global topology of cardiac communication networks derived from single-nuclei transcriptomic data. CellChat-inferred networks from healthy controls (Day 0), EAM mice at the DCM stage (Day 40), and metformin-treated EAM mice (Day 40, metformin administered from day 17 in a therapeutic mode) were modelled as weighted directed graphs comprising 13 cardiac cell types and 74 signalling pathways. Pathway-level communication probabilities were transformed into 697-dimensional graph representations and analyzed using a pathway-resolved graph kernel support vector machine (SVM) with leave-one-out cross-validation. Distinct condition-specific communication structures were identified, with the model achieving 75% multiclass classification accuracy, significantly exceeding chance levels (33.3%; permutation test, $p = 0.006$). Healthy myocardium was classified with 100% accuracy, demonstrating that EAM produces a globally detectable restructuring of cardiac communication topology. In contrast, binary classification between untreated and metformin-treated EAM networks failed (50% accuracy), indicating that metformin does not fundamentally rewire disease-associated communication architecture but instead modulates signaling intensity within a preserved network framework. Node ablation analysis identified cardiac microvascular endothelial cells and mural cells as key structural determinants of disease-state classification. Collectively, these findings demonstrate the utility of graph-based machine learning for systems-level modeling of intercellular communication dynamics and therapeutic mechanisms in inflammatory cardiomyopathies.

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Machine Learning of Slow Modes: RNA Pseudoknots and Ligand Binding

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Long-timescale processes such as protein folding and chemical reactions are often characterized by several metastable states separated by high-energy barriers. Sampling of these systems is hindered by the limited timescales accessible in traditional molecular dynamics simulations. This limitation can be addressed using enhanced sampling techniques. Enhanced sampling techniques aim to accelerate rare events and enable more comprehensive exploration of conformational space. Several of these methods require a few meaningful degrees of freedom, known as collective variables (CVs), before simulation. Optimal CV selection plays a key role in ensuring efficient enhanced sampling. Conformational changes occur on longer timescales, and optimal CVs are associated with the system's slow modes. Conventional methods are widely used, but CVs obtained this way may not capture the system's slow modes. Therefore, machine learning (ML) techniques have recently been proposed specifically to address the problem of identifying slow CVs.

In this work, we present a spectral map method, a novel, unsupervised learning approach capable of finding thermodynamically informed slow modes, for the extraction of slow CVs from high-dimensional molecular dynamics data [1]. The spectral map method separates slow and fast variables by maximizing the spectral gap of a Markov transition matrix constructed from simulation data. We show how spectral map can be used on an especially complex system: RNA pseudoknot bound with inhibitors [2].

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Functional genomic characterization of selenium-related pathways in Lactiplantibacillus plantarum isolate

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Selenium (Se) is essential for antioxidant defense and immune function, yet deficiency remains widespread due to a narrow safety range and poor bioavailability of inorganic Se species. Se nanoparticles (SeNPs) offer a promising alternative with reduced toxicity, enhanced bioactivity, and food industry potential, especially via microbial production using lactic acid bacteria (LAB). In this scenario, this study aimed to evaluate the selenium metabolism potential of four milk and cheese-derived Lactiplantibacillus plantarum isolates, ML7, ML99, CH131, and CH135, using an in silico approach based on whole-genome functional annotation. Genome sequencing was performed on the Illumina NextSeq 1000 platform, with gene prediction conducted using Prodigal software. Genes involved in selenium uptake, transport, and detoxification pathways were identified and manually curated in the four genomes. Comparative genomic analysis revealed differences in oxidative stress-related pathways. The isolate CH131 is the only strain candidate that lacks glutathione peroxidase (gpx), indicating a reduced capacity to eliminate reactive oxygen species (ROS) generated during selenite reduction. Although CH131, together with the ML7 isolate, has more copies of DNA-binding ferritin-like protein (dps), showing an enhanced Fenton reaction prevention caused by Se-generated H_2O_2. Furthermore, L. plantarum ML7 uniquely harbored the complete operon involved in Se-induced proteotoxic stress (clpABCLPX), indicating a high maintenance against damage caused by selenium exposure. These genomic differences suggest that isolate ML7 has greater genetic potential for selenium reduction and tolerance. However, gene presence does not guarantee functional expression, and in vitro validation studies are essential to confirm the actual capacity of these isolates for SeNP synthesis.

Transcriptomic Profiling of miRNA-Mediated Hepatoprotection by Silybum marianum against Aflatoxin B1-Induced Toxicity in Pigs

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Background: Aflatoxin B1 (AFB1), a potent hepatotoxin, significantly disrupts liver function by interfering with RNA regulatory networks. While the hepatoprotective properties of Silybum marianum (SM) are well-recognized, the specific role of microRNA (miRNA) modulation in this process remains poorly defined.

Methods: In this study, a feeding experiment was conducted with 154 pigs to investigate the transcriptomic shifts induced by SM supplementation. One cohort received a standardized diet supplemented with SM (90 mg/kg BW), while the control group received the AFB1-contaminated diet only. Liver RNA libraries were sequenced and analyzed to identify differentially expressed miRNAs (DEmiRs) and their functional implications.

Results: Transcriptomic profiling revealed a distinct signature of miRNA dysregulation in the SM group compared to the Control. Following a stringent filtration process (Padj < 0.15), nine significant DEmiRs were identified. The expression profile demonstrated a bi-directional regulatory pattern: 66.7% of the core DEmiRs were upregulated, while 33.3% were downregulated. The most statistically robust finding was the significant downregulation of ssc-miR-148b-5p (Padj = 0.000875; logFC = -0.821), indicating a substantial de-repression of its downstream metabolic targets. Conversely, ssc-miR-34a was intensely upregulated (logFC = 1.741), alongside the synchronized over-expression of the evolutionarily conserved miR-99/100 family (ssc-miR-99a-5p, ssc-miR-99b, and ssc-miR-100). Functional enrichment and in silico target prediction revealed that these dysregulated miRNAs converge on the PI3K/Akt/mTOR signaling axis, suggesting a targeted, repressive modulation of this growth and metabolism pathway as a primary mechanism of SM-mediated protection.

Conclusion: These findings indicate that SM exerts substantial hepatoprotective effects against AFB1-induced toxicity through the highly coordinated miRNA-mediated regulation of immune and metabolic pathways. The identification of shared miRNA signatures highlights potential universal molecular targets for mitigating mycotoxin-induced liver damage in swine.

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Integer Programming Model for RNA Secondary Structure Prediction: Efficient Multiloop Representation

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Predicting RNA secondary structure from its primary sequence is a fundamental and computationally challenging task in bioinformatics. It is often solved with variants of dynamic programming (DP), which are very efficient for standard energy-based models. However, extending these methods with new structural constraints or experimental data can be difficult and will require nontrivial modifications to the underlying recursion. Here we present an integer linear programming (ILP) formulation for RNA secondary structure prediction that is based on the same nearest-neighbor energy approach and naturally supports integration of more complex structural or experimental constraints.

In the proposed ILP model, structural elements, such as stems, hairpins, internal loops, and multiloops, are encoded through binary decision variables and linear constraints. We also introduce a scalable representation of multiloops, which are among the hardest aspects of RNA structure modeling. To avoid explicit multiloop enumeration and improve computational tractability, multiloops are incorporated into the model via their constituent internal and closing branches. The model determines the optimal secondary structure by minimizing the objective function, which represents the total free energy of the structure as the sum of all feasible loop energy contributions. The optimization process is delegated to general-purpose ILP solvers.

The model was tested on sequences of up to 120 nucleotides from the Archive II benchmark dataset. The predicted structures achieve accuracy comparable to classical DP methods. The branch-and-cut algorithm used by ILP solvers explores multiple feasible intermediate structures before the optimal solution is found, thereby enabling the identification of suboptimal structures. It was observed that for some sequences these structures match the references better than the optimal ones. Although computationally more expensive than DP algorithms, the ILP approach can produce solutions for RNA sequences of biologically relevant length. These results demonstrate the potential of the proposed model for constrained and hypothesis-driven RNA structure prediction.

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Transcriptomic Profiling of Andrographis paniculata Treatment reveals a Dual miRNA-mRNA Regulatory Network Modulating Inflammation, Apoptosis, and Fibrosis in a Porcine Model

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Background: Andrographis paniculata (AP) is a renowned medicinal plant recognized for its immunomodulatory, antiviral, and anti-inflammatory properties. However, the precise post-transcriptional mechanisms driving its therapeutic efficacy remain fully elucidated. This study investigated the microRNA (miRNA) regulatory landscape following AP administration to identify key molecular axes governing its bioactive response.

Methods:A transcriptomic analysis was conducted using a porcine (Sus scrofa) model to evaluate differential miRNA expression between AP-treated and control groups. Computational algorithms (TargetScan, miRanda, and RNAhybrid) were employed to predict downstream mRNA targets based on 3' UTR binding stability. Network interaction mapping was used to assess the regulatory breadth and determine "one-to-many" or "many-to-one" interactions between differentially expressed miRNAs (DEMs) and their target genes. Results: We identified 23 significantly upregulated DEMs and a distinct cohort of downregulated DEMs in the AP-treated group. Among the upregulated miRNAs, ssc-miR-186-5p, ssc-miR-19a, and ssc-miR-19b emerged as "Master Regulators," each targeting over 2,000 genes. Functional enrichment of these targets revealed systemic modulation of apoptosis (BCL2, AKT2), immune signaling (TLR10), and viral entry, highlighted by the targeting of ACE2 by miR-186-5p. Furthermore, the simultaneous upregulation of the miR-30 family (a–e) suggests an anti-fibrotic role via the inhibition of epithelial-to-mesenchymal transition (EMT). Conversely, critical downregulated miRNAs, including ssc-miR-146a-5p, ssc-miR-181a, and ssc-miR-150, indicated a coordinated release of gene inhibition. Network analysis highlighted TRAF6 as a central hub targeted by both miR-146a-5p and miR-181a, suggesting potent modulation of the TLR and NF-κB inflammatory pathways. Other key targets included SMAD4 (TGF-β signaling) and HMGCR (cholesterol metabolism), altogether favoring the activation of anti-inflammatory and cytoprotective genes such as SOCS3, TRAF3, and PPARG.

Conclusion: These findings demonstrate that the therapeutic effects of Andrographis paniculata are mediated by a sophisticated dual-regulatory miRNA network. By simultaneously upregulating master regulators of tissue integrity and downregulating microRNAs that suppress anti-inflammatory/metabolic hubs (like the miR-146a/181a-TRAF6 axis), AP exerts a systemic, multi-pathway stabilizing effect. This dataset establishes a definitive framework for future integrative miRNA-mRNA validation studies.

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Utilizing QM/MM and Molecular Orbital Methods to Explore The Mechanism of the Catalytic Oxidation of SAPE by 15LOX-1 in a complex with PEBP1

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The catalytic oxidation of polyunsaturated fatty acids-phosphatidylethanolamine (PUFA-PE) is a key biochemical event in the initiation and progression of ferroptosis—an iron-dependent, non-apoptotic form of regulated cell death [1]. Ferroptosis has been implicated in numerous pathological conditions, including neurodegenerative diseases, ischemia-reperfusion injury, and chronic inflammatory disorders such as Cystic Fibrosis [2-4]. It has been shown that a protein complex of 15-lipoxygenase (15LOX) and PE-binding protein 1 (PEBP1) cause PUFA-PE transformation into its oxidized form [5]. Understanding the mechanism of PUFA-PE oxidation by 15LOX-1/PEBP1 complex is essential for identifying molecular targets for drug development. In this work, we apply hybrid quantum mechanics/molecular mechanics (QM/MM) methods to elucidate the reaction pathway, revealing key intermediates and transition states. Additionally, we present a generalizable computational workflow for investigating a broad range of iron-based enzymatic systems involved in lipid peroxidation and redox biology.

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RNAmoley: spatially resolved analysis and refinement of RNA 3D models

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The assessment of RNA 3D structure predictions relies predominantly on global quality metrics, both in method development and in community-wide evaluation initiatives such as RNA-Puzzles and CASP. While these measures are effective for ranking models, they provide limited insight into the local sources of structural inaccuracies. As predictive methodologies - including AI- and data-driven approaches - continue to advance, there is a growing need to identify specific regions and structural motifs that are systematically mispredicted or affected by geometric and stereochemical errors.

Here, we present RNAmoley, a web-based framework for spatially resolved analysis and targeted refinement of RNA 3D models. The method introduces a neighborhood-oriented strategy based on a walking sphere algorithm, enabling precise localization of structural irregularities across the model or within user-defined regions. This approach allows detection of local issues such as steric clashes, backbone conformational anomalies, and stereochemical inconsistencies that remain obscured at the global level. RNAmoley integrates these analyses with interactive 2D and 3D visualizations, facilitating intuitive exploration of RNA structures at multiple levels of detail. In addition, the platform enables targeted correction of local defects through short restrained energy minimization, supporting iterative model improvement.

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Non-native Bio-amidation Activity in Asparagine Synthetase for the Synthesis of (S)-2-Aminobutyramide

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Chiral amides represent cornerstone motifs in pharmaceuticals, yet their sustainable synthesis remains challenging due to a scarcity of natural enzymes capable of direct amidation. Here, we report the engineering of a non-native bio-amidation activity within Escherichia coli asparagine synthetase A (AsnA) for the single-step synthesis of (S)-2-aminobutyramide ((S)-2-AB), a critical intermediate for anti-epileptic drugs levetiracetam or brivaracetam. Through directed evolution, we generated a variant (K77L/E120L/R255W) that achieves an inversion of substrate specificity, converting (S)-2-aminobutyric acid with high stereoselectivity. Docking studies and molecular dynamics (MD) simulations reveal that these mutations fundamentally remodel the active site, inducing a 180° flip in the substrate's binding mode. This structural reorientation positions the non-native carboxyl group in a catalytically productive geometry relative to ATP – a conformation that remains entirely inaccessible in the wild-type enzyme.

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Transcriptomic Analysis of Curcuma Treatment Effects using In Vitro experiment on Porcine Hepatocytes cell culture : A Preliminary MultiOmics Study

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Background: Curcuma, a bioactive compound derived from turmeric, has demonstrated diverse biological activities, including anti-inflammatory, antioxidant, and metabolic regulatory effects. However, the comprehensive transcriptomic response to Curcuma treatment in porcine hepatocyte systems remains incompletely characterized.

Methods: We performed microRNA-seq analysis comparing control (Group A, n=3) versus Curcuma-treated (Group B, n=3) in vitro porcine (Sus scrofa) hepatocytes. Differential gene expression analysis was conducted using DESeq2, with significance thresholds set at adjusted p-value < 0.05 and |log2 fold change| > 1. Functional enrichment analysis was performed using Gene Ontology (GO) and pathway databases (KEGG, Reactome).

Results: Of 1,953 genes tested, 390 showed significant differential expression (padj < 0.05), with 99 genes upregulated and 57 genes downregulated in Curcuma-treated cells (|LFC| > 1, padj < 0.05). Curcuma treatment induced robust heat shock protein expression (HSPA6: LFC = +5.16, padj = $1.53 \times 10^{-}12$; HSPB1: LFC = +1.67), upregulated lipid metabolism genes (APOA4, RBP4, TM7SF2, OSBPL2), and downregulated metabolic regulators (GCLC: LFC = -2.29, padj = 6.32×10^{4}; ANP32B: LFC = -2.15, padj = 2.53×10^{-16}. Functional enrichment revealed significant modulation of cholesterol efflux (padj = $1.11 \times 10^{-}4$), complement and coagulation cascades (padj = 3.43×10^{-9}), and translational regulation pathways.

Conclusions: Curcuma treatment elicits a multifaceted transcriptomic response characterized by cellular stress adaptation, lipid metabolic reprogramming, immune pathway activation, and suppression of gluconeogenesis. These findings provide molecular insights into Curcuma's pleiotropic biological effects and suggest potential therapeutic applications in metabolic and inflammatory disorders.

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Elucidating the Epigenetic Landscape of Curcuma: MicroRNA-mRNA Regulatory Networks Modulate Inflammation and Lipid Metabolism in a Porcine Model

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Background: Curcuma is a prominent bioactive phytogenic additive widely utilized in animal nutrition for its anti-inflammatory, antioxidant, and metabolic-regulating properties. This study aimed to characterize the comprehensive microRNA (miRNA) expression profile altered by Curcuma supplementation and identify the downstream transcriptomic networks responsible for its therapeutic efficacy.

Methods: Differential miRNA expression profiling was conducted comparing a Curcuma-treated (Sus scrofa) cohort against a control group. Downstream messenger RNA (mRNA) targets and biological networks were computationally predicted and validated utilizing the miRNet database, integrating experimentally verified and predicted interaction repositories (including miRTarBase, TarBase, and TargetScan). Network topology analysis was utilized to determine regulatory "hubs" based on target multi-connectivity and gene-centric multimodal regulation.

Results: Curcuma supplementation induced a massive transcriptomic reorganization. A total of 76 miRNAs were significantly upregulated, collectively targeting 7,608 unique genes. Top master regulator, ssc-miR-2320-5p, alone targeted 2,044 genes, followed by ssc-miR-195 (1,029 targets) and ssc-miR-221-5p (1,010 targets). Pathway analysis revealed strategic post-transcriptional silencing of primary inflammatory cascades: 66 of the 76 miRNAs targeted the TNF signaling family, while over 60% targeted the core drivers of cellular stress and inflammation, namely the NF-κB and MAPK pathways. Downregulated Network. Conversely, 83 miRNAs were downregulated. However, key master-regulatory "brakes," specifically the let-7 (notably ssc-let-7g and ssc-let-7e) and miR-16 families, were highly active, leading to the severe, multimodal down-regulation of crucial downstream metabolic and inflammatory hub genes. Key suppressed targets included SCD, a major regulator of lipid metabolism; TRAF3, a crucial mediator of pro-inflammatory signaling; ESR1, involved in hormonal and growth signaling; and AKT3/MAP3K1, central to cellular proliferation.

Conclusion: These results demonstrate that Curcuma exerts its health-promoting benefits via a sophisticated, dual-action epigenetic shift in a porcine model. By simultaneously upregulating a broad spectrum of miRNAs that silence systemic inflammatory pathways (TNF, NF-κB, and MAPK) and executing targeted multi-miRNA suppression of specific metabolic and signaling hub genes (such as SCD and TRAF3), Curcuma effectively shifts the cellular phenotype toward a highly protected anti-inflammatory and lipid-lowering state.

Acknowledgements: This research is financed and supported by the scientific project NCN-OPUSLAP (UMO-2021/43/I/NZ9/02612) entitled: Multilevel molecular analysis of the hepatoprotective effect of medicinal herbs extracts in prevention of liver dysfunction caused by aflatoxin B1 in pig as an animal model (in-vivo), and hepatocyte cell culture analysis in human and pig (in-vitro).

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Transcriptomic Orchestration by Silymarin: Unveiling the MicroRNA-mRNA Regulatory Network in a Porcine Model

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Background: Silymarin, an extract derived from milk thistle (Silybum marianum), is widely recognized for its hepatoprotective, antioxidant, and anti-inflammatory properties. While its phenotypic benefits are well-documented, particularly in porcine models, the underlying post-transcriptional regulatory mechanisms remain fully elucidated. This study aimed to map the microRNA (miRNA) expression profile altered by Silymarin treatment and decode its downstream transcriptomic networks to understand its broad-spectrum therapeutic action.

Methods: Differential miRNA expression analysis was performed comparing a Silymarin-treated (Sus scrofa) group against a Control group. Significantly altered miRNAs were identified, and their downstream mRNA targets were computationally predicted and validated using the miRNet platform (integrating miRTarBase, TarBase, and TargetScan). Network topology analysis was employed to identify high-impact "hub" miRNAs based on unique target gene counts.

Results: Silymarin administration induced a bidirectional transcriptomic shift, significantly modulating two distinct regulatory networks:

• Upregulated Network: A total of 28 miRNAs were significantly upregulated. The top 20 master regulators included ssc-miR-221-5p and ssc-miR-204, each targeting over 1,700 genes, and ssc-miR-340. Together, these upregulated miRNAs targeted 3,662 unique genes, suggesting a systematic suppression or "braking" of transcripts involved in pro-inflammatory signaling, lipid accumulation, MAPK, and PI3K-Akt pathways.

• Downregulated Network: Conversely, 35 miRNAs were significantly downregulated, resulting in the potential "de-repression" (upregulation) of 7,112 unique target genes. Key regulatory hubs identified included ssc-miR-185 (2,314 targets), ssc-miR-15b (1,970 targets), and ssc-miR-29a-5p (1,923 targets). These miRNAs are critically linked to apoptosis, cell cycle regulation, and extracellular matrix/fibrosis pathways.

Conclusion: Our findings demonstrate that Silymarin does not merely target isolated genetic pathways but rather orchestrates a comprehensive transcriptomic response in a porcine model. By simultaneously upregulating miRNAs that repress pathological processes (such as inflammation and lipid accumulation) and downregulating miRNAs that restrict survival pathways (favoring anti-fibrogenesis and cellular survival), Silymarin exerts its multi-faceted hepatoprotective effects via highly connected miRNA-mRNA hub networks.

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Revealing hidden substrate patterns via Gamma-Secretase proteome clustering

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Gamma-Secretase (GS) is a membrane-bound protease most widely recognized for its role in Alzheimer's disease. This multi-subunit protein complex cleaves the amyloid precursor protein (APP), leading to the production of amyloid-beta 42, which aggregates to form the amyloid plaques characteristic of the disease. However, therapies targeting GS have shown severe side effects, primarily because the biology of GS is significantly more complex than just processing APP - GS cleaves over 200 different substrates that do not share a single common sequence motif. Recently, new techniques to identify substrates were developed, and many novel interactions were discovered. The goal of this work was to update the previously established list of substrates with these latest discoveries and cluster them by employing ESM-2 embeddings combined with an Agglomerative Clustering algorithm, followed by bioinformatic analysis to identify group-specific commonalities. This analysis will help clarify and predict side effects in GS-targeted therapies, ultimately supporting the future design of new, more selective drug candidates.

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RNAbridge: a database of extended and non-canonical helices

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RNA 2D structure based on 3D coordinates is usually studied by considering only non-pseudoknotted, canonical base pairs. If provided and supported, pseudoknots and non-canonical interactions are added later and analyzed only in the previously defined context. Unfortunately, this method can mask the fact that straight helices, as seen in 3D, can be composed of several stems connected by bulges or loops, which are usually rich in non-canonical interactions. As a result, these longer non-standard helices are missed altogether, even though their presence marks important RNA 3D architectural constraints and should be acknowledged by RNA 3D structure prediction methods.

Here, we introduce RNAbridge, the first tool developed to collect and organize extended non-standard helices. The database identifies almost 75,000 such helices in RNA 3D structures deposited in the Protein Data Bank, many of which occur as coaxially stacked components of otherwise already complex junctions. RNAbridge offers interactive views, filtering options, clear summaries, and download choices for dedicated visualizations and 3D data of specific motifs. Weekly automatic updates keep the database current with new structural information, and ready-made examples make it easy to explore.

Understanding extended non-standard helices is important for correctly predicting RNA 3D structures, assessing modeling accuracy, and comprehending how complex junctions are arranged in space. RNAbridge offers a key database to help progress RNA structural biology and computer-based structure prediction. The website is free for everyone to use with no login needed at https://rnabridge.cs.put.poznan.pl/.

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Workshops

Mastering RAG: customizing Large Language Models for bioinformatics

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The volume of biological data, from genomic sequences to clinical trial reports, often outpaces the ability of researchers to synthesize information manually. While Large Language Models (LLMs) offer a transformative way to interact with this data, they are prone to hallucinations and lack access to private patient data. This workshop introduces Retrieval-Augmented Generation (RAG) as a robust architecture for solving these challenges. Participants will learn how to ground LLMs in specific biological knowledge bases, build local RAG pipelines to ensure data privacy, and move beyond 'generic' AI to create specialized tools for drug discovery, protein analysis, and personalized medicine. The workshop will cover vector database integration, embedding models specialized for biological text and the deployment of local models to ensure HIPAA/GDPR compliance. Participants will leave with a functional prototype of a RAG system tailored to query complex biological datasets.

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