CMS core facilities are operated by the Institute of Biotechnology, Czech Academy of Sciences. The Centre of Molecular Structure is supported by: Czech Infrastructure for Integrative Structural Biology (CIISB), Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042) and OP JAK project „Innovation of Czech Infrastructure for Integrative Structural Biology“(No. CZ.02.01.01/00/23_015/00081 75).

Thursday, March 19, Session II

L4

EFFICIENT ENRICHMENT OF *IN CELLULO* GROWN PROTEIN CRYSTALS

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Among the well-established methods for protein crystallization, *in cellulo* crystallography has a special place, as it allows for investigation of protein structure in the cellular milieu. However, due to limits in current understanding of why some proteins undergo spontaneous ordered assembly, *in cellulo* crystallization has remained a niche technique to date. Many protein targets that can be identified *in cellulo* crystallization are inaccessible for X-ray diffraction experiments due to insufficient yields of these crystals in cell culture. Here, we introduce a novel approach for enriching cells that harbor *in cellulo* crystals. We employ linked co-expression of a marker protein and *in cellulo* crystallizable protein target to obtain a direct correlation between the marker protein's signal and the crystallization probability of the target. We demonstrate this approach on established *in cellulo* crystallization targets HEX-1 from *N. crassa* and cathepsin B from *T. brucei*, by sorting subpopulations of crystal-containing cells from the cell culture. This technique is part of the *in cellulo* crystallization pipeline used by EMBL at the PETRA III beamlines to identify novel in cellulo crystallization targets, to improve the concentration of crystal-containing cells, and to prepare cryo-samples for serial synchrotron diffraction data collection in a robust and reliable manner. This study presents necessary innovations with the potential to make the *in cellulo* protein crystallography more available for structural biology studies of proteins without known experimental structures in the intricate cellular environment.

This work was supported by the MEYS CR (LUAUS25250).

L5

INSIGHT INTO GABA SHUNT-ASSOCIATED ALDEHYDE DEHYDROGENASES (ALDH) AND STRESS RESPONSES OF ALDH SUPERFAMILY IN MOSS AND BARLEY

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Aldehyde dehydrogenases (ALDHs) play a central role in detoxifying reactive aldehydes generated during catabolism, or lipid peroxidation. Disruption of ALDH-dependent pathways challenges cellular redox balance and necessitates activation of compensatory detoxification mechanisms [1]. As a first step, we performed a comparative analysis of ALDH superfamily expression in two distinct plant models, a moss P*hyscomitrium patens* and barley (*Hordeum vulgare*) under diverse stress conditions.

Beside their role in oxidative stress adaptation, many ALDH families also participate in diverse metabolic pathways. Building on this, our work complements current knowledge of GABA shunt-associated ALDHs using structural and kinetic approaches and resolves the first plant ALDH5 structure, a missing piece of the puzzle. Us-

ing *P. patens* knockouts lacking key GABA shunt–associated ALDHs, we combined a biochemical characterization with in vivo approaches, including phenotyping, metabolite profiling, and whole-transcriptome RNA-seq analysis, to investigate metabolic and detoxification responses to impaired aldehyde oxidation. Moss knockouts revealed functional redundancy between ubiquitous ALDH5 and moss-specific ALDH21. Notably, six distinct glutathione *S*-transferase genes were strongly upregulated in all aldh mutants, uncovering a novel metabolic crosstalk between ALDH-dependent aldehyde oxidation, GST-mediated detoxification, and the GABA shunt pathway. This highlights a robust and flexible network that preserves redox homeostasis and metabolic balance under impaired aldehyde metabolism [2].

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This work was supported by Junior grant no. JG_2020_001 and IGA_PrF_2025_011 from Palacký University, the JAK project 'TowArds Next GENeration Crops' (no.885CZ.02.01.01/00/22_008/0004581) from the Ministry of Education, Youth and Sports of the Czech886Republic (MEYS CR), and the Jean d'Alembert fellowship as part of France 2030 program ANR-11-887IDEX-0003. This work benefited from the I2BC crystallization platforms, supported by FRISBI ANR-88810-INSB-05-01.

L6

MOLECULAR BIOPHYSICS DATABASE FOR ANNOTATED RAW DATA

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With the number of methods of molecular biophysics growing every year, there are still very limited resources for storage of raw data with annotation of experimental conditions for their reuse.

The Molecular Biophysics Database (MBDB) stores raw data files together with metadata descriptions. The design of the database makes measurement results **F**indable, **A**ccessible, **I**nteroperable and **R**eusable.

The metadata for individual data sets consist of a general part and a method-specific part. The general part defines, in a unique way, descriptors for key parameters common for different experimental techniques, e.g. source organism, identity of individual molecules, including chemicals, with reference to external databases and unique identifiers for the most relevant types. The method-specific part is devoted to the metadata special for a particular technique (such as MST, BLI, etc.). This approach enables searching across different techniques and allows direct comparisons of results, e.g. interaction parameters, for the same molecular system measured by different techniques.

The Molecular Biophysics Database (MBDB) is built using the Invenio repository platform technology (https://inveniosoftware.org/) and JSON as the key representation format of metadata, in collaboration with the CESNET data storage team and their hardware resources.

MBDB was launched in January 2025 (https://mbdb-data.org/) and is ready for deposition of raw measurement files with rich metadata describing experimental details for the techniques MST, BLI, SPR and ITC [1]. We invite researchers to deposit their raw data and make them publicly available, with each record receiving a DOI identifier.

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We acknowledge support by the Horizon 2020 programme of EU (MOSBRI, no. 101004806) and by MEYS (CIISB support, LM2023042 and the OP JAK programme – project NRP, no. CZ.02.01.01/00/23_014/0008787).

L7

BASE PAIRING? BASE PAIRING!

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Base pairing is the fundamental property governing the structural and functional landscape of nucleic acids. While Watson-Crick (W-C) pairs facilitate genetic information storage and form the scaffold of RNA, they represent only a fraction of the structural diversity. Understanding the complex architecture of RNA and the non-canonical arrangements of DNA is impossible without a rigorous description of non-W-C interactions [1].

Using the Leontis-Westhof classification schema [2], all possible pair topologies can be systematically described via three base edges: Watson-Crick, Hoogsteen, and Sugar. By combining these edges in *cis* or *trans* orientations, we arrive at twelve base pair families (cWW, tWW, cWH, tWH, cWS, tWS, cHH, tHH, cHS, tHS, cSS, tSS). When accounting for base identity across the four standard nucleotides, these expand into 156 unique pair classes, such as the ubiquitous cWW_G-C or the rarer tWH_A-G, tWH_G-A, cHS_A-U, …

Currently, automated programs for pair assignment, unfortunately including the one utilized by the PDB, often yield controversial, incomplete, or incorrect results. To address this, we developed a new algorithm based on transparent parameter definitions and geometric distributions that define the limits of all possible pairs [3].

In this presentation, I will introduce our design, testing, and implementation of this algorithm, explaining our protocols for pair assignment and validation. I will provide a statistical overview of pair distributions across diverse RNA structures from the PDB and demonstrate our web service, DNATCO (dnatco.datmos.org) [4]. Specifically, I will highlight three unique DNATCO features:

- NAPASCO: Our validation metric for gauging base pair quality.
- Interactive 3D Rendering: Graphical visualization of all pair classes within any annotated structure.
- Interoperability: How we capture these complex interactions into newly developed mmCIF dictionaries.

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This research was funded by Czech Academy of Sciences, grant RVO 86652036 and by grant LM2023055.

L8

BETTER SAMPLES, BETTER STRUCTURES: NEW PRACTICAL TOOLS FOR CRYO-EM AND CRYSTALLOGRAPHY

Paul Driver

Molecular Dimensions, Cambridge

High-quality structural data begins with high-quality samples, yet many structural biology projects are limited by recurring challenges in sample preparation rather than instrumentation. In this talk, **Molecular Dimensions** will present several recently introduced products designed to improve robustness, reproducibility, and cost-effectiveness across cryo-EM and crystallography workflows.

A major focus will be a new vitrification screen for cryo-EM grid preparation. This systematic screening approach is designed to mitigate common issues such as air–water interface interactions, protein aggregation, and poor particle dispersion, helping researchers achieve more uniform grids and improved data collection outcomes.

We will also introduce a new protein concentrator that delivers performance comparable to established market leaders, while offering a significantly reduced cost of ownership. This provides a practical alternative for routine protein concentration during sample preparation and optimisation.

In addition, the talk will highlight recently launched crystallisation screens, including dedicated organic and alcohol screens, developed to expand chemical space and improve crystallisation success for challenging targets.

The session will conclude with brief closing remarks from **Pharmatech**, who will introduce a new FPRC system and outline its relevance within modern structural biology laboratories.

L9

FROM EXPRESSION TO INTERACTION: BIOPHYSICAL CHARACTERIZATION OF RNA-PROTEIN COMPLEXES ACROSS THE STRUCTURAL BIOLOGY WORKFLOW

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RNA-protein interactions lie at the heart of fundamental biological processes - from gene regulation and splicing to RNA modification and interference mechanism. Comprehensive characterization of these interactions remains a significant challenge, often requiring complex biophysical approaches demanding high sample consumption and tedious optimization at each stage of the research.

Here we present an integrated biophysical workflow addressing the complexity of issues related to RNA-protein interactions and the challenges they pose from recombinant protein expression monitoring and quality control, through thermal and colloidal stability assessment, to quantitative binding affinity determination. At each stage with sensitivity and reproducibility for reliable results.

Starting from the beginning Andromeda X enables real-time monitoring of protein expression, supporting construct optimization and ensuring sufficient yields of target protein before downstream characterization begins. Prometheus Panta enables rapid thermal and colloidal stability screening of full-length and truncated protein constructs, providing essential quality control data prior to structural studies. Monolith and Dianthus deliver quantitative RNA-protein binding affinity measurements in solution, with minimal sample consumption and no immobilization required - capturing even weak or transient interactions with high sensitivity.

The presented research-based examples illustrate how comprehensive biophysical characterization based on individual NanoTemper systems facilitates and accelerates measurement and parameterization, spanning from expression monitoring, through stability characterization, to affinity measurements – helping researchers solve problems precisely at the stage of the workflow where they are most blocking.