XXII Discussions in Structural Molecular Biology and 9th User Meeting of the Czech Infrastructure for Integrative Structural Biology

Annual Meeting of the Czech Society for Structural Biology

Conference Centre of the Czech Academy of Sciences, Nové Hrady, March 19 - 21, 2026

Organisers:

Jan Dohnálek, Jarmila Dušková, Jan Stránský, Kristýna Adámková, Radek Kužel

The event is organized by the Czech Society for Structural Biology, the Czech Infrastructure for Integrative Structural Biology, and the Institute of Biotechnology of the Czech Academy of Sciences.

Thursday, March 19, PhD Thesis Award 2025

L1

STRUCTURE-FUNCTION STUDIES OF TUBULIN-MODIFYING ENZYMES

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Microtubules (MTs) exhibit high genetic diversity due to the existence of multiple - and -tubulin isoforms, encoded by distinct genes with tissue-specific expression patterns. MT functional properties are further diversified by post-translational modifications (PTMs), forming a complex regulatory system known as the tubulin code.

The thesis aims to investigate the structure and function of tubulin tyrosine ligase-like 11 (TTLL11), an enzyme with marked polyglutamylase activity. TTLL11 modifies not only tubulin but also other physiological substrates, including Dishevelled 3 (DVL3) from the Wnt signaling pathway. To elucidate the substrate specificity and catalytic mechanism of the enzyme, we evaluated a series of TTLL11 variants for their capacity to bind and polyglutamylate tubulin *in vitro* and in a cellular environment. Our findings revealed that, while TTLL11 shares sequence similarity with other TTLL enzymes exclusively within its catalytic domain, its C-terminal region is indispensable for function.

Using cryo-electron microscopy, we uncovered a unique interaction pattern between TTLL11 and MTs, wherein the TTLL11 MT-binding helix bundle (MT-BHB) engages with one protofilament, while its catalytic domain extends over an adjacent protofilament. Importantly, we discovered a novel tubulin post-translational modification, wherein the C-terminus of the primary tubulin polypeptide undergoes direct elongation, facilitating the re-entry of the 2 and 3 tubulin variants, previously regarded as "dead-end" variants, into the tubulin modification cycle. Notably, substrate preferences were not driven by a tubulin isotype but rather by the physicochemical properties of the C-terminal amino acid with strong preference for glutamate. These findings expand our understanding of the tubulin code by introducing a previously unrecognized dimension of tubulin tail elongation, further highlighting the role of TTLL11 in cytoskeleton regulation.

Thursday, March 19, Session I

L2

AN INTRODUCTION TO INSTRUCT-ERIC: FUNDED ACCESS TO STRUCTURAL BIOLOGY INFRASTRUCTURE

Pauline Audergon

Instruct-ERIC Hub, Oxford House, Parkway Court, John Smith Drive, Oxford OX4 2JY

Instruct-ERIC is a pan-European distributed research infrastructure making high-end technologies and methods in structural biology available to users. Instruct-ERIC is comprised of 17 Member Countries and organisations: Belgium, Czech Republic, EMBL, Finland, France, Germany, Greece, Israel, Italy, Latvia, Lithuania, Netherlands, Portugal, Slovakia, Slovenia, Spain and United Kingdom. Through its 12 specialist research centres in Europe, Instruct-ERIC offers funded research visits, training, internships and R&D awards. Access is available to researchers worldwide, with funding available to researchers in Instruct's Members, including Czechia. Instruct also hosts the Instruct Biennial Structural Biology Conference, the next edition will be taking place in Brussels in May 2026. By promoting integrative methods, Instruct-ERIC enables excellent science and technological development for the benefit of all life scientists.
More on https://instruct-eric.org/

L3

CENTRE OF MOLECULAR STRUCTURE – PROTEIN PRODUCTION AND TECHNOLOGIES FOR STRUCTURAL AND BIOPHYSICAL ANALYSIS

M. Alblová, T. Černovec, J. Pavlíček, P. Pompach, J. Plucarová, J. Stránský, J. Dohnálek, B. Schneider, M. Huličiak

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The Centre of Molecular Structure (CMS), part of the Czech Infrastructure for Integrative Structural Biology (CIISB) and the Instruct-ERIC centre is located at the Institute of Biotechnology of the Czech Academy of Sciences in Vestec. CMS provides comprehensive services for studying the three-dimensional structure, function, and biophysical properties of biological molecules. CMS consists of several specialized core facilities (CF).

The Protein Production CF supports the entire pipeline from DNA to purified protein, including gene cloning and expression in diverse E. coli strains or in eukaryotic expression systems (HEK293T, Expi293 and Expi293F™ GnTI- mammalian cells, or insect cells such as Sf9 and High Five™), followed by multistep purification. Users can order any stage of the process separately or the whole process as a single package.

Biophysical Methods CF provides techniques for the characterization of biomolecular interactions (e.g. ITC, MST, SPR, BLI, switchSENSE) and determination of the size, mass, structure, and stability of biomolecules and their complexes (e.g. CD, DSF, MADLS, and MP).

The CF for Crystallization of Proteins and Nucleic Acids focuses on obtaining crystals of biomacromolecules and their complexes. It offers both manual and robotic setting of crystallization experiments under various conditions and automated monitoring of crystal growth in crystallization hotels (with remote web access).

The Diffraction Techniques CF offers structural characterization of proteins via single crystal X-ray diffraction and small-angle X-ray scattering for liquid samples analysis.

The Structural Mass Spectrometry CF enables high-resolution studies of intact proteins, protein-protein interactions, proteomics, and metabolomics.
The Data management CF is a new facility opened in 2026. Our vision is that it will help to deal with data flow within CMS and the whole IBT and contribute to our stride to open science principles.

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

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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.

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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-