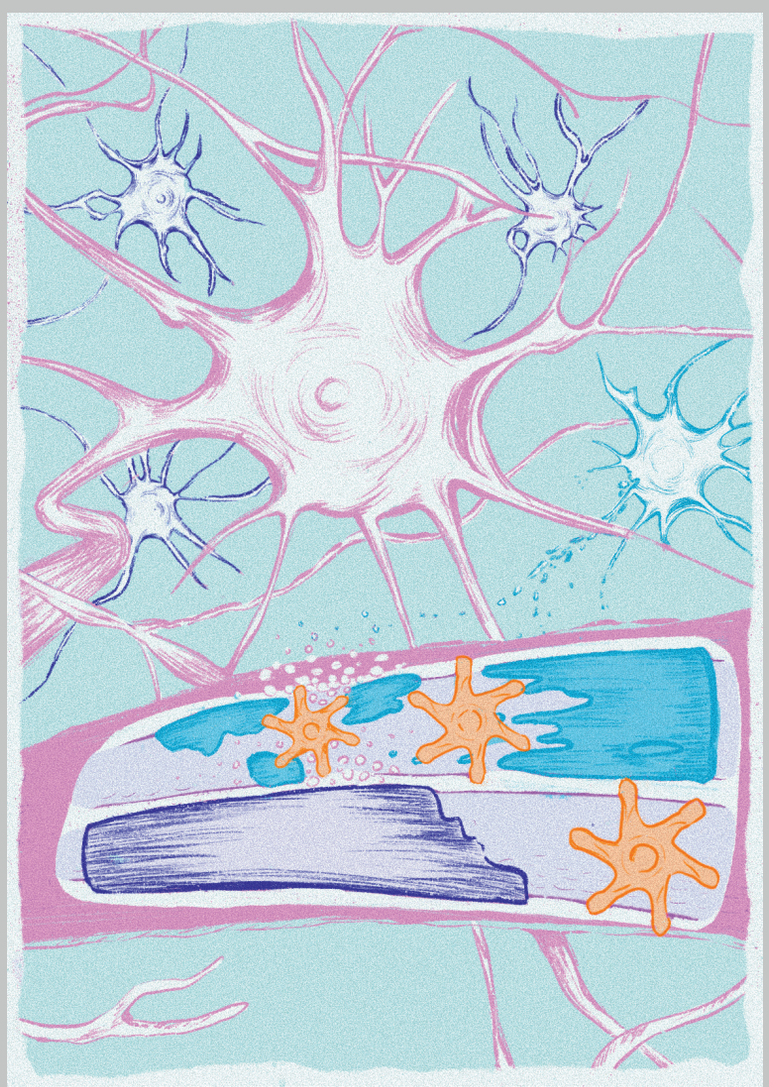
MATERIALS STRUCTURE
in Chemistry, Biology, Physics and Technology



Czech and Slovak Crystallographic Association

vol. 31, no. 1, 2025

MATERIALS STRUCTURE

in Chemistry, Biology, Physics and Technology

Bulletin of the Czech and Slovak Crystallographic Association

www.xray.cz

Editorial notes

The issue contains the abstracts of all contributions presented at the 21st meeting of the Czech and Slovak Structural Biologists named “Discussions in Structural Molecular Biology” and the 8th CIISB user meeting - 29 lectures and 51 posters. For more information about the conference please see the web pages http://cssb.structbio.org.

General notes for authors

Different kinds of contributions are accepted - articles on the problems of atomic and real structure of materials, general papers on crystallography, review articles, book reviews, short reports about conferences, schools and seminars, information on X-ray and neutron laboratories, new instrumentation, computing methods and programs, letters to the editors and short messages. Two independent referees review the papers and review papers. The complete manuscript (electronic version) in English, Czech or Slovak with figures and tables must be delivered to one of the editors. Different text processors can be used for the text preparation. RTF text format can be exported from nearly any text processor and this is suitable also for editorial office. Please, look at the exact format of the references and keep it. English abstract and keywords must be placed at the beginning of each paper. The correct citation of the journal is Materials Structure.

The Web page of the journal is http://www.xray.cz/ms. Please, see it for more details.

On cover page: The image on the cover was designed by Anna Karhanova.
It was taken from Valerie Siahaan's PhD thesis receiving the best PhD thesis of 2024 award by the Czech Society for Structural Biology.

Supported by the Czech Academy of Sciences
Published by the Czech and Slovak Crystallographic Association (CSCA).
Technical editors: Ivana Kutá Smatanová, Radomír Kužel.
Supported by the Czech Academy of Sciences.
Printed by Karel Hájek, designhhstudio.
ISSN 1211 5894 (print), ISSN 1805-4382 (Online) volume **31** (March 2025), no. 1 @CSCA

XXI Discussions in Structural Molecular Biology
and
8th User Meeting of CIISB

20-22 March 2025

Programme

Thursday, March 20

12:00 - 14:00 Registration and accommodation, coffee

All talks are 20 minutes long unless indicated otherwise. All times are INCLUDING discussion.

14:30 **Opening of XXI Discussions and 8th User Meeting of CIISB** (*Jan Dohnálek)*
Remembering Vladimír Sklenář *(Lukáš Žídek)*

14:45 - 15:30 **Session I** *Chair person:* **Jan Dohnálek** *Lecture no. – page of abstract*

Valerie Siahaan Institute Curie, CNRS, Orsay, France	Tau proteins cooperatively assemble into cohesive envelopes that protect microtubules against severing enzymes (PhD Thesis Award 2024- winner's talk, 30 min)	*L1 – 6*

15:30 - 16:00 *Coffee break*

16:00 - 18:00 **Session II** *Chair person:* **Lukáš Žídek**

Stefana Njemoga Institute of Neuroimmunology, Slovak Academy of Sciences	Sequence-based polymorphism of Tau protein amyloid fibrils (Student talk)	*L2 – 7*
Martin Černý Masaryk University, CEITEC, Brno	RNA polymerase subunit delta - elusive player in bacterial transcription (Student talk)	*L3 – 8*
Miroslav Kloz ELI Beamlines, Dolní Břežany	Photoactivation of Human Green Cone Opsin Studied by Stimulated Raman Spectroscopy: Initial Steps Toward Understanding the Early Events in Human Vision	*L4 – 9*
Edward Curtis Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague	Development and application of catalytic DNA sensors	*L5 – 10*
Jiří Zahradník First Faculty of Medicine, Charles University, Prague	Chlamydia Effector CT622/TaiP Complex with Autophagy Master Regulator ATG16L1	*L6 – 10*
Jakub Nowak NanoTemper Technologies Sp.	Beyond Monolith X- The principles of Spectral Shift (10 min)	*L7 – 11*
Josef Uskoba BioTech a.s., Prague	Use of FIDA for rapid characterization of Liquid-Liquid Phase Separation (10 min)	*L8 – 11*

18:00 Dinner

20:00 ***CIISB Executive committee***

Friday, March 21

7:30 - 9:00 *Breakfast*

9:00 - 10:30 **Session III** *Chair person:* **Vojtěch Spiwok**

Jan Dohnálek Institute of Biotechnology, Czech Academy of Sciences, Vestec	Can sequence-, structure- and sugar-nonspecific nucleases be harnessed? ***L9 – 13***
Jakub Bělíček Palacký University, Olomouc	Oligomerization as a Regulatory Mechanism in Plant Adenosine Kinase Activity (Student talk) ***L10 – 14***
Jana Horáčková Masaryk University, Faculty of Science, Brno	Shedding light on the secrets of NanoLuc, its mechanism, and allosteric behaviour (Student talk) ***L11 – 15***
Miroslav Peřina Palacký University, Olomouc	CDK2-based CDK7 mimic as a tool for structural analysis: Biochemical validation and crystal structure with SY5609 ***L12 – 16***
Paul Driver Molecular Dimensions, Cambridge	Crystallizing the Future: Unveiling New Innovations at Molecular Dimensions (10 min) ***L13 – 17***

10:30 - 11:00 *Coffee break and group photo*

11:00 - 12:10 **Session IV** *Chair person:* **Jana Škerlová**

Maša Janošev Institute of Physiology, Czech Academy of Sciences, Prague	Structural Mechanisms of Regulation of Human Nedd4-2 by 14-3-3η dimer and Calcium ions (Student talk) ***L14 – 18***
Martin Malý Medical Research Council, Cambridge	*Servalcat* and *MetalCoord*: New structure refinement strategies in *CCP4 and CCP-EM* ***L15 – 19***
Hynek Mácha ANAMET s.r.o., Prague	From characterization of proteins and their interactions to their determination in nanocarriers ***L16 – 20*** (10 min)

Poster flash talks, 2 minutes each

Ondřej Bulvas (Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague)
Dynamic Allosteric Regulation of Mycobacterial Inosine Monophosphate Dehydrogenase

Miroslava Alblová (Institute of Biotechnology, Czech Academy of Sciences, Vestec)
Protein Production Facility – DNA & Proteins for Your Research

Vladěna Bauerová (Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava)
The effect of the cardiac-associated mutations on the biophysical properties, fold and structure of the N-terminal domain of human ryanodine receptor 2

Mateo Seoane Blanco (Masaryk University, CEITEC, Brno)
PhiKZ baseplate structure

Yelyzaveta Pulnová (ELI Beamlines, Dolní Břežany)
Laser-driven plasma X-ray source at ELI Beamlines (Student poster)

Sahra Setenay Baran (Institute of Biotechnology, Czech Academy of Sciences, Vestec)
Improved validation and refinement of biomolecular structures (Student poster)

Jitka Vysloužilová (University of South Bohemia, Faculty of Science, České Budějovice)
Structure and Dynamic Properties of Porphyrin Aggregates in Solution (Student poster)

Martin Sitte (Masaryk University, Faculty of Science, Brno)
Functional characterisation of the luminous apparatus of the sea pen *Pennatula phosphorea* (Student poster)

Jayashri Bhosale (Charles University, Faculty of Science, Prague)
Structural insights into 14-3-3-mediated regulation of human ASK1 (Student poster)

12:30 *Lunch*

16:00 - 17:50 **Session V** *Chair person:* **Edward Curtis**

Vojtěch Spiwok University of Chemistry and Technology, Prague	Amino Acids in the Context of Protein Structure: What we Can Learn from Protein Large Language Models	*L17 – 21*
Hugo McGrath University of Chemistry and Technology, Prague	Gating of the ribosome exit tunnel (Student talk)	*L18 – 21*
Gowtham Nirmal Jonnalagadda University of South Bohemia, Faculty of Science, České Budějovice	Long-Range Electron Transfer in Protein-Metal Junctions (Student talk)	*L19 – 22*
Jakub Hrubý Ecole Polytechnique Federale de Lausanne	High-resolution Liquid Cells for Time-resolved Cryo-EM (Student talk)	*L20 – 23*
Klára Kohoutová Charles University, Faculty of Science, Prague	Structural Basis of the 14-3-3/Cyclin Y-mediated Regulation of CDK16 (Student talk)	*L21 – 24*
Stanislav Kukla Merck Life Science, Prague	Seeing is believing: Proximity ligation assay (10 min)	*L22 – 25*

18:00 - 19:00 **General Assembly of the Czech Society for Structural Biology**

The meeting will be held in the Czech and/or Slovak languages

1. Volba zapisovatele
2. Schválení programu
3. Volby Rady ČSSB na období 2025-2030
4. Volba Kontrolní komise ČSSB na období 2025-2030
5. Výroční zpráva Rady ČSSB o činnosti a hospodaření
6. Zpráva revizní komise
7. Schválení výroční zprávy Rady ČSSB o činnosti a hospodaření
8. Revize členské základny
9. Infrastruktury se vztahem ke strukturní biologii, přístupy, financování, vývoj Instructu a jiných
10. Různé

19:00 - 20:00 *Dinner*

20:00 - 22:00 **Poster session,** (CSSB Council meeting)

Saturday, March 22

7:30 - 9:00 *Breakfast*

9:00 - 10:20 **Session VI** *Chair person*: **Jiří Pavlíček**

Gabriel Demo Masaryk University, CEITEC, Brno	Cryo-EM analysis of *E. coli* ribosome recovery mechanism in the absence of the 30S maturation factor RimM	*L23 – 26*
Jiří Nováček Masaryk University, CEITEC, Brno	Snapshots of the glucose metabolism studied by electron cryo-microscopy *in vitro* and *in situ*	*L24 – 26*
Michaela Novotná Institute of Microbiology, Czech Academy of Sciences, Prague	CLINCELIN: A redesigned lincosamide combats ribosome resistance modification through enhanced binding and structural flexibility (Student talk)	*L25 – 27*
Jana Škerlová Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague	Structure of botulinum-like toxins	*L26 – 27*

10:20 - 10:50 *Coffee break*

10:50 - 11:50 **Session VII** *Chair person:* **Michaela Wimmerová**

Ladislav Bumba Institute of Microbiology, Czech Academy of Sciences, Prague	*In situ* visualization of the *Bordetella* filamentous hemagglutinin by cryo-electron tomography	*L27 – 29*
Alastair Gardiner Institute of Microbiology, Czech Academy of Sciences, Prague	Two solutions for efficient light-harvesting in phototrophic *Gemmatimonadota*	*L28 – 29*
Pavel Plevka Masaryk University, CEITEC, Brno	Cell entry and genome delivery of enteroviruses	*L29 – 30*

11:50 - 12:20 **Evaluation of student presentations, prizes, concluding remarks**

12:30 *Lunch & departure*

XXI Discussions in Structural Molecular Biology and 8th User Meeting of the Czech Infrastructure for Integrative Structural Biology

Meeting of the Czech Society for Structural Biology

Conference Centre of the Czech Academy of Sciences, Nové Hrady, March 20 - 22, 2025

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 20, Session I

PhD Thesis Award

L1

Tau PROTEINS COOPERATIVELY ASSEMBLE INTO COHESIVE ENVELOPES THAT PROTECT MICROTUBULES AGAINST SEVERING ENZYMES

V. Siahaan[1,2], M. Braun[1], Z. Lansky[1]

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[2]Current address: Institut Curie, Université PSL, CNRS UMR3348, Orsay, France
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Tau is a microtubule-associated protein that is preferentially found in the neuronal axons. In neurodegenerative diseases, collectively termed tauopathies, malfunction of tau and its detachment from axonal microtubules, often associated with abnormal phosphorylation of tau, are correlated with axonal degeneration and loss of microtubule mass [1]. Tau can protect microtubules from microtubule-degrading enzymes such as katanin [2] and regulate transport by molecular motors along the microtubule [3,4]. However, how tau carries out these regulatory functions is still unclear. Using in vitro reconstitution and TIRF microscopy, we show that tau molecules can bind to microtubules in two distinct modes: either as (i) single tau molecules independently diffusing on the microtubule surface, or (ii) cooperatively-bound tau that form a cohesive tau "envelope" enclosing the microtubule lattice [5-8]. We found that tau envelope formation alters the spacing of tubulin dimers within the microtubule lattice, where envelope formation compacted the underlying lattice, and lattice extension induced tau envelope disassembly [7]. Tau envelopes form a selectively permissible barrier that inhibits kinesin-1 motors while allowing dynein movement, and protects microtubules against the activity of microtubule severing enzymes such as katanin [5]. Tau envelopes itself are regulated by tau phosphorylation, where phosphorylation of tau leads to destabilization of "healthy" non-phosphorylated tau envelopes and reduced protective functionality of the envelopes [8]. Combined, our data reveals the microtubule-dependent cooperative binding mode of tau that can constitute an adaptable protective layer on the microtubule surface. The subtle change in the microtubules lattice structure can differentially affect the affinities of other microtubule-binding proteins to the microtubule surface, thus potentially dividing microtubules into functionally distinct segments. Finally, our data suggests that a reduction in microtubule mass linked to tau hyperphosphorylation in neurodegenerative diseases, could be explained by the destabilization and impaired functionality of the tau envelopes upon tau phosphorylation.

1. A. Kneynsberg et al., *Front Neurosci*, **11**, (2017), 572.

2. L. Qiang et al., *The Journal of Neuroscience*, **26**(12), (2006), 3120–3129.
3. M. Vershinin et al., *Proceedings of the National Academy of Sciences*, **104**(1), (2007), 87–92.
4. R. Dixit et al., *Science*, **319**(5866), (1979), 1086–1089.
5. V. Siahaan et al., *Nat Cell Biol*, **21**(9), (2019), 1086–1092.
6. R. Tan et al., *Nat Cell Biol*, **21**(9), (2019), 1078–1085.
7. V. Siahaan et al., *Nat Chem Biol*, **18**(11), (2022), 1224–1235.
8. V. Siahaan et al., *Nat Chem Biol*, (in review).

Thursday, March 20, Session I

L2

SEQUENCE-BASED POLYMORPHISM OF Tau PROTEIN AMYLOID FIBRILS

S. Njemoga[1], Z. Bednarikova[2], E. Barrera[3], Z. Gazova[2], O. Cehlar[1]

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[3]IHEM, Universidad Nacional de Cuyo (CONICET), Mendoza, Argentina
stefana.njemoga@savba.sk

Thanks to advancements in cryo-electron microscopy, the structure and sequence composition of tau amyloid fibrils from different diseases are well-established [1]. However, the exact mechanisms underlying disease-specific mechanisms, spreading patterns, and resulting disease-specific fibrils morphology remain elusive, hindering the development of specific anti-tau therapies. Previously validated amyloid motifs in the tau protein sequence are regarded as carriers of tau amyloidogenicity because of their intrinsic beta propensity and ability to assemble into stable amyloid fibrils core [2-3]. Together with environmental factors and posttranslational modifications (PTMs), these short amyloid-nucleating motifs contribute to the formation of different protofilaments interface, which underpin the amyloid fibrils polymorphism. One of the most common PTMs that plays a key role in the pathogenesis of Alzheimer's disease is truncation, which promotes the self- assembly of tau monomers by exposing regions that are prone to aggregation in Alzheimer's disease [4]. In some cases, however, truncations in amyloid-prone regions can impair further amyloid aggregation. In our study, five variants of different lengths of tau involving different amyloid motifs were used, including two experimentally tested PHF6(306-311) and PAM4(350-362) and predicted G(326-331) and L(341-449) to demonstrate the sequence-dependent aggregation mechanism. All tau variants were truncated at residue 391 from the C-terminus and at the different sites from N-terminal part of the protein (297-391, 306-391, 316-391, 321-391, 326-391). Propensity toward amyloid aggregation of five tau variants was evaluated by ThT fluorescence assay, and the presence of polymorphic amyloid fibrils was confirmed by atomic force microscopy under four aggregation conditions – with or without heparin and DTT. Tau321-391 exhibits the highest amyloid robustness across all tested conditions, being the only tau variant to produce filaments under physiological conditions represented by pure PBS buffer. On the other hand, tau326-391 variant failed to produce the fibrils even in the presence of heparin, indicating the crucial role of 321-325 for tau monomers self-assembly.

Molecular dynamics simulations revealed an increased propensity of 321-325 sequence toward beta-structures in all atom simulations but a rather helical propensity in coarse-grained simulations. Tau321-391 monomers interacted through a helical interface, which was already featured for other amyloid proteins in early stages of aggregation. MD simulations successfully replicated sequence-specific beta-sheet propensity in agreement with computationally predicted and experimentally established amyloid-nucleating motifs.

1. A. Fitzpatrick, B. Falcon, S. He et al., *Nature*, **547**, (2017), pp. 185-190.
2. M. von Bergen, P. Friedhoff, J. Biernat et al., *P.N.A.S.*, **97**, (2000), 5129.
3. N. Louros, M. Wilkinson, G. Tsaka et al., *Nat. Commun.*, **15**, (2024), 1028.
4. B. Kovacech and M. Novak, *Current Alzheimer Research*, **7**, (2010), pp. 708-716.

Part of the research results was obtained using the computational resources procured in the national project National competence centre for high perFormance computing (project code:311070AKF2) funded by European Regional Development Fund, EU Structural Funds Informatization of society, Operational Program Integrated Infrastructure. This research was funded by the European Union's Horizon Europe program under the grant agreement No. 101087124. This work was also funded by the grants APVV 21-0479, APVV-22-0598 and Vega 2/0125/23, 2/0141/23 and 2/0141/25. This research has received funding from the European Union's Horizon 2020 research and Innovation programme under the Marie Skłodowska-Curie grant agreement No 873127. This research was further funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00623.

L3

RNA POLYMERASE SUBUNIT DELTA - ELUSIVE PLAYER IN BACTERIAL TRANSCRIPTION

Martin Černý[1,2], Viktor Bartošík[2], Klára Mikesková[3], Hana Šanderová[3], Krásný Libor[3], Žídek Lukáš[1,2]

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[2]Central European Institute of Technology, Masaryk University, Kamenice 5, Brno 62500, Czech Republic
[3]Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology of the Czech Academy of Sciences, v.v.i., Vídeňská 1083, Prague 4 14220, Czech Republic
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Since the 80s, the δ-subunit is in the spotlight of many researchers who tried to solve the mystery of its function. It was first described in *Bacillus subtilis*. However, it became apparent that it is part of the transcription machinery in many *Firmicutes*, like *Staphylococcus aureus* or *Streptococcus pyogenes*. It is also present in intracellular bacterial parasites from class *Mollicutes*. δ-subunit is associated with the bacterial RNA polymerase (binding partner of its subunit β'), where it plays a crucial role in transcription initiation and termination. It was demonstrated that it serves a role of major transcription regulator of genes, that are responsible for environmental adaptation, virulence and sporulation [1,2,3,4].

Structural studies showed that the δ-subunit is roughly 20 kDa large protein, consisting of 2 domains. N-terminal globular domain which binds to the RNA polymerase core, and highly acidic and intrinsically disordered C-terminal one. While binding of the N-terminal domain to the RNA polymerase was more or less established, the structure and binding of the C-terminal domain was solved relatively recently by H. H. Pei [3,4,5]. However, current findings shed light only on the transcription termination and recycling of the RNA polymerase complex, while the mechanism and function of the C-terminal domain remain obscured.

In the past, our group structurally characterized the δ-subunit of *B. subtilis*, and identified an important sequential and structural feature - the lysine tract. We investigated and confirmed, that the negatively charged C-terminal domain interacts with the tract, creating a more compacted structure of the whole protein [6]. In this study, we aimed to describe the relation between the length of the C-terminal domain and the effect of the lysine tract on the transcription and the structure of the δ-subunit. To probe the possible effects, we compared it to its homolog from *S. aureus*, which naturally lacks the lysine tract. Furthermore, we characterized the *S. aureus* δ-subunit structure using SAXS and NMR, which gave us some important clues about the nature and function of this regulatory protein.

1. Williamson V. M., Doi R. H., Delta factor can displace sigma factor from *Bacillus subtilis* RNA polymerase holoenzyme and regulate its initiation activity. *Molec. Gen. Genet.* 161, 135–141 (1978). https://doi.org/10.1007/BF00274183
2. Xue X., Tomasch J., Sztajer H., Wagner-Döbler I., The Delta Subunit of RNA Polymerase, RpoE, Is a Global Modulator of *Streptococcus mutans* Environmental Adaptation. *J Bacteriol* 192, (2010). https://do9b07837i.org/10.1128/jb.00653-10
3. Rabatinová A., Šanderová H., Jirát Matějčková J., *et al*., The δ Subunit of RNA Polymerase Is Required for Rapid Changes in Gene Expression and Competitive Fitness of the Cell. *J Bacteriol* 195, (2013). https://doi.org/10.1128/jb.00188-13
4. Pei H. H., Hilal T., Chen Z. A. *et al.* The δ subunit and NTPase HelD institute a two-pronged mechanism for RNA polymerase recycling. *Nat Commun* 11, 6418 (2020). https://doi.org/10.1038/s41467-020-20159-3
5. Yuan L., Liu Q., Xu L. *et al.* Structural basis of promoter recognition by *Staphylococcus aureus* RNA polymerase. *Nat Commun* 15, 4850 (2024). https://doi.org/10.1038/s41467-024-49229-6
6. Kubáň V., Srb P., Štégnerová H., *et al*. Quantitative Conformational Analysis of Functionally Important Electrostatic Interactions in the Intrinsically Disordered Region of Delta Subunit of Bacterial RNA Polymerase. *J Am Chem Soc*. 141, 42:16817-16828 (2019). https://doi.org/10.1021/jacs.

L4

PHOTOACTIVATION OF HUMAN GREEN CONE OPSIN STUDIED BY STIMULATED RAMAN SPECTROSCOPY: INITIAL STEPS TOWARD UNDERSTANDING THE EARLY EVENTS IN HUMAN VISION

Miroslav Kloz[1], Sarah Luise Schmidt[2], and Polina Isaikina[2]

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[2]Laboratory of Biomolecular Research, Paul Scherrer Institute PSI, Switzerland

Retinal proteins have been the focus of scientific investigation for several decades, leading to a substantial body of knowledge about these pivotal components of the biosphere. However, despite this progress, detailed insights into the dynamics of opsins underlying human vision remain remarkably limited [1]. This knowledge gap arises not from a lack of interest but from significant challenges inherent in the study of these proteins. Firstly, the eukaryotic nature of human opsins renders their preparation highly complex. Secondly, their monostable nature causes the proteins to undergo irreversible disintegration following a single photon absorption. A collaborative effort between biochemists from the Paul Scherrer Institute and metrologists from the ELI Beamlines facility has successfully addressed these challenges. Employing approximately 50 microliters of a human green opsin sample, the researchers captured the photoisomerization process with a high signal-to-noise ratio. This achievement represents the first-ever recorded dynamics of human cone opsins. These findings constitute a significant advancement in our understanding of human vision and provide a foundation for comparative studies with other retinal-driven photobiological processes. Current results suggest surprisingly large differences in the structure and dynamics of cone opsins responsible for color vision compared to the somewhat better-understood rhodopsins that mediate achromatic night vision.

1. Gerd G. Kochendoerfer, Zhiyan Wang, Daniel D. Oprian,and Richard A. Mathies, Biochemistry, **36**, 6577-6587 (1997).

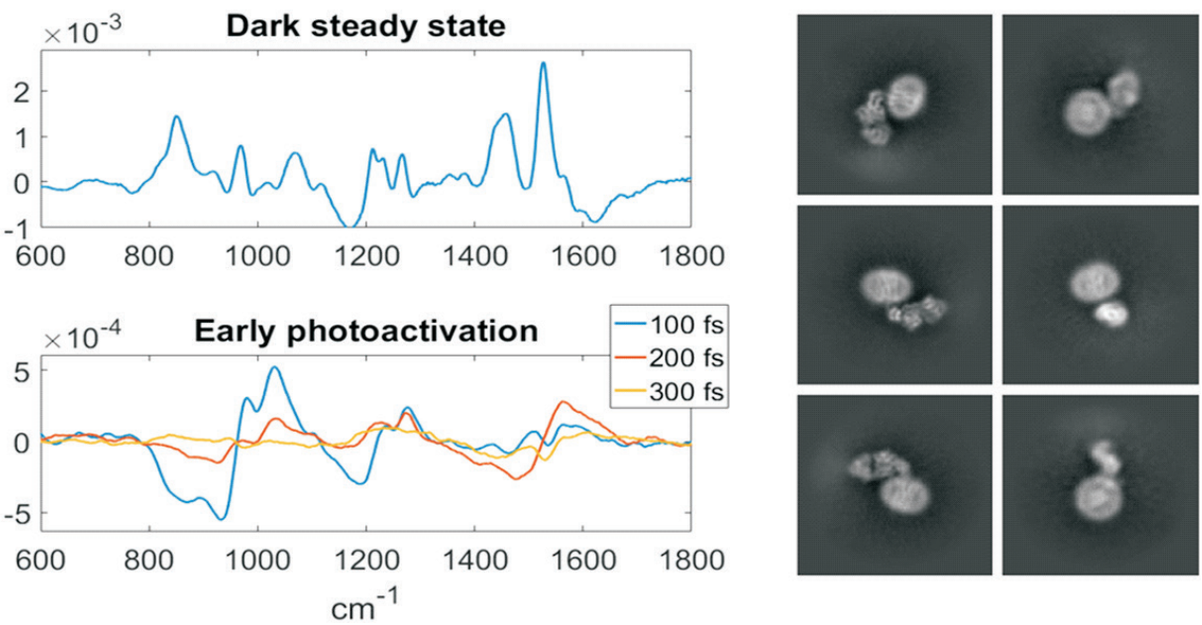

Figure 1. Steady state, time resolved femtosecond stimulated Raman spectra and cryo-EM images of green opsin. Time resolved dynamic was after photoactivation at 560 nm. Results suggests that isomerization is almost fully completed after 200 fs making it even faster than in bovine rhodopsin and arguably the fastest biochemical reaction ever observed.

L5

DEVELOPMENT AND APPLICATION OF CATALYTIC DNA SENSORS

Martin Volek, Jaroslav Kurfürst, Kateřina Švehlová, Matúš Drexler, Michal Svoboda, Martin Jakubec, Milan Kožíšek, Pavel Srb, Václav Veverka, Edward Curtis

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo náměstí 542/2, Prague 6, 160 0, Czech Republic

People typically think of DNA as a molecule that stores genetic information, but this remarkable polymer can also have many other functions. These include the ability to catalyze chemical reactions. In this presentation I will talk about catalytic DNA molecules recently developed in our group that generate chemiluminescent, fluorescent, and colorimetric signals. I will also discuss our efforts to convert these DNA enzymes into sensors that only generate a signal in the presence of specific target molecules. Such sensors have great potential for applications such as diagnostics and high-throughput screening.

L6

CHLAMYDIA EFFECTOR CT622/TaiP COMPLEX WITH AUTOPHAGY MASTER REGULATOR ATG16L1

Adam Hruška[1], Aditi Konar[1], Martin Mokrejš[1,2], Petr Kolenko[2], Jiří Zahradník[1]

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[2] *Institute of Biotechnology CAS, BIOCEV, Prague 252 50, Czechia*
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Chlamydia trachomatis is an obligate intracellular bacterium that relies on a diverse arsenal of secreted effector proteins to manipulate host cellular processes and establish the inclusion, a specialized compartment essential for its proliferation. Among these effectors, CT622/TaiP has emerged as a key regulator of host-pathogen interactions, yet its precise molecular function remains elusive.

Previous findings indicated that CT622/TaiP directly interacts with the WD40 domain of ATG16L1[1], a crucial autophagy regulator involved in autophagosome formation and multiple protein interactions. As part of our collaborative project with Prof. Agathe Subtil (Institut Pasteur), we aimed to characterize this complex and propose a potential

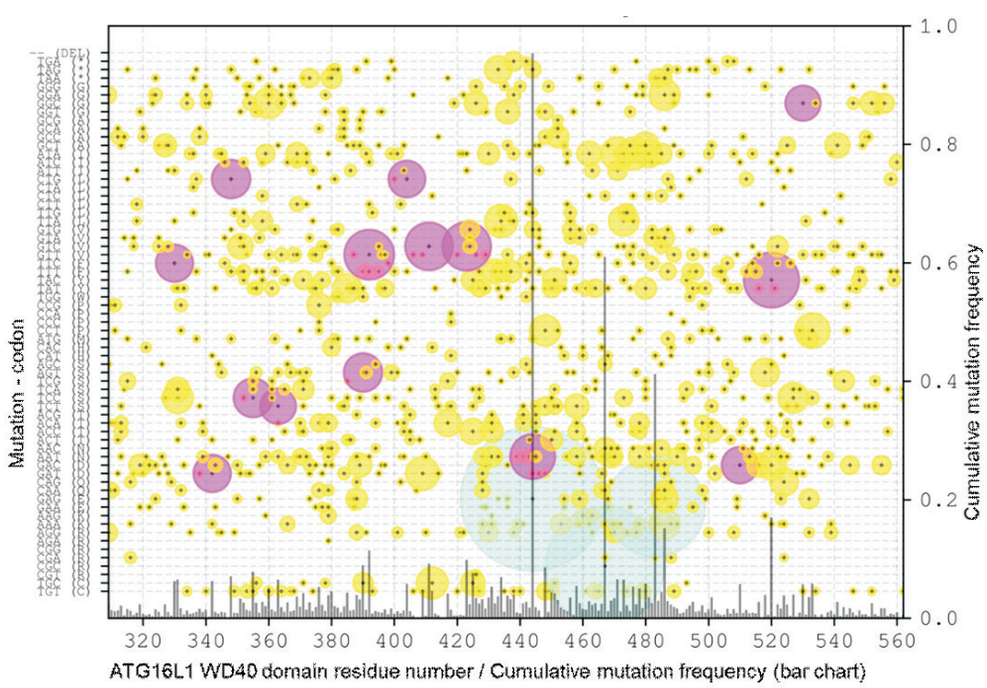

Figure 1. Results of yeast display-based mapping of the CT622/TaiP–ATG16L1 binding site and affinity maturation. Circle size represents mutation frequency within the selected population. Color code: sky blue for mutations with frequency >0.2, magenta for mutations with frequency >0.05 and yellow for mutations with frequency bellow 0.05

mechanism by which *Chlamydia* modulates autophagy to evade degradation.

Determining the experimental structure of this complex presented a significant challenge due to the unfavorable biochemical properties of both proteins, necessitating extensive protein engineering to enhance their stability and affinity. Since predictive algorithms such as AlphaFold-Multimer failed to accurately model the interaction, we employed yeast display-based mapping of the binding site and affinity maturation (Figure 1). To support our mutational data, we utilized hydrogen–deuterium exchange mass spectrometry and mutational analyses, which allowed us to design protein variants sufficiently stable for cryo-EM structure determination.

Our findings provide new insights into the molecular basis of CT622/TaiP–ATG16L1 binding and establish a framework for understanding how *Chlamydia* manipulates the autophagy machinery, with potential implications for therapeutic intervention.

1. Hamaoui D., et al. (2020) The Chlamydia effector CT622/TaiP targets a nonautophagy related function of ATG16L1, Proc Natl Acad Sci U S A. 2020.

„The project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.“

L7

BEYOND MONOLITH X- THE PRINCIPLES OF SPECTRAL SHIFT

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Among many existing fluorescence-based applications, dedicated to characterization of molecular interactions, vast majority depend on site-specific labeling, binding- induced change of conformation, or size of interacting molecules. To overcome these limitations, we applied a ratio-m etric dual-emission approach that quantifies ligand-induced spectral shifts with sub-nanometer sensitivity. The use of environment-sensitive near-infrared dyes with the method we describe, enables affinity measurements and thermodynamic characterization without the explicit need for site-specific labeling or ligand-induced conformation changes. The newest isothermal spectral shift technology, implemented together with TRIC (temperature related intensity change), in newest NanoTemper system, Monolith X allows researchers to work in solution with variety of biomolecules, including proteins, antibodies, and nucleic acids, as well as with the most challenging types of targets, like membrane or intrinsically disordered proteins.

L8

USE OF FIDA FOR RAPID CHARACTERIZATION OF LIQUID-LIQUID PHASE SEPARATION

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Liquid-Liquid Phase Separation (LLPS) is a phenomenon caused by the spontaneous and reversible formation of condensates that results in a highly concentrated dense phase and a dilute phase [1].

In some cases, liquid to solid transitions occur, causing the formation of amyloid fibrils, amorphous aggregation, and gelation. Even though sometimes beneficial to the cell, these events are mostly associated with detrimental effects related to various neurological disorders such as ALS, Alzheimer's and Parkinson's disease [5]. As a result, LLPS has gained increased attention in academic and industrial settings [6]. Despite this, the field is lacking easily approachable methods for rapid characterization of the key parameters.

This Application Note is based on the paper of Stender, Ray & Norrild et al. published in Nature Communication [3]. It describes how FIDA is used as the new method to rapidly characterise multiple crucial LLPS parameters using μL of sample with no need of prior expertise in the technology.

Using Fida 1 as the single experimental platform, we measured dilute phase concentrations, droplet count, relative droplet size distribution, kinetics of droplet formation, maturation into amyloid fibrils as well as the affinity between proteins undergoing LLPS and LLPS-modulating compounds.

More specifically, we analysed the influence of ssDNA on the condensation of the n1 domain of human DEAD-box helicase 4 (Ddx4n1). Ddx4n1 is a protein involved in creating the nuage in egg and sperm cells and is well known for its role in partitioning polynucle-otides [7]. We also present how FIDA is used to study the liquid to solid transition of α-synuclein into Thioflavin T positive amyloid fibrils - a process involved in Parkinson's disease-

showing the great potential of the technology for the study of LLPS-related neurological disorders [8].

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Friday, March 21, Session III

L9

CAN SEQUENCE-, STRUCTURE- AND SUGAR-NONSPECIFIC NUCLEASES BE HARNESSED?

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Fungi, trypanosomatids, plants and some pathogenic bacteria code for 3'-nucleases/nucleotidases which belong to the S1-P1 nuclease family [1]. While for example in *Leishmania amazonensis* its membrane-attached 3'-nucleotidase is suspected to be a virulence factor, in plants they have a role in growth and cell death. The role of bacterial enzymes from this family is not clear.

S1-P1 nucleases are metalloenzymes of MW ~25-40 kDa, typically zinc-dependent, relying on a cluster of three divalent metals in a surface cavity of an almost fully α-helical fold. The cluster, binding the phosphate group after the cleaved bond, is accompanied by the nucleobase-binding site 1 which is responsible for stabilization of the −1 nucleotide with respect to the cleaved O3'-P3' bond. These enzymes can act as endonucleases and nucleotidases. They cleave DNA, RNA, single strands, double strands, viroids, some modified nucleotides, oligonucleotides and genomic DNA, substrates which are structure or unstructured, without any significant sequence preference. These properties make them ideal candidates for biotechnological applications [1].

Interestingly, even if their fold basically does not change across the biological species, their activity profiles can differ dramatically, in some cases being basically a ss DNase with negligible activity towards double strands, in some cases more of an RNase, and for example in plants showing comparable activity towards all types of nucleic acids. Some eukaryotic representatives (tomato) require glycosylation to maintain the enzyme stable, while the bacterial versions with very close structure are stable at high concentrations without such modifications.

We have studied S1-P1 nucleases from plants, fungus, and two bacterium species [2-5]. Crystallographic studies, mutagenesis, numerous product/ligand complexes helped us better understand the structure-function questions, such as active site remodelling, sensitivity to metal replacement, key mobility elements in the active site and more. Recently, for the first time we have identified a "supernuclease" capable of previously unseen rates for this enzyme class, uncovered the key region for RNA/DNA preference, which opens door to "tailor-made" optimization, and discovered its high activity towards cyclic-di-GMP, the bacterial second messenger [6]. The crystallization properties of some representatives enable studies at high resolutions, making it possible for us to start asking questions about protonation states of enzyme and its substrate upon encounter.

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This work was supported by the Czech Science Foundation (25-17546S), and by the Czech Academy of Sciences (86652036). CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project No. CZ.02.01.01/00/23_015/0008175 is acknowledged for providing access to all facilities at CMS in BIOCEV for this project.

L10

OLIGOMERIZATION AS A REGULATORY MECHANISM IN PLANT ADENOSINE KINASE ACTIVITY

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Adenosine kinase (ADK) catalyses ATP-dependent phosphorylation of adenosine and cytokinin ribosides in plants. Ado is primarily synthesized through the activity of S-adenosyl homocysteine (SAH) hydrolase [1] and purine nucleoside phosphorylase (PNP) catalysing Ado ribosylation [2]. Since SAH hydrolase participates in the S-adenosyl methionine (SAM) cycle, the accumulation of Ado can lead to its feedback inhibition, thereby disrupting the cycle and impairing SAM-dependent transmethylation. To prevent this, ADK role in removing Ado is pivotal [3].

We examined the substrate preferences, oligomeric states, and structures of ADKs from moss (*Physcomitrium patens*) and maize (*Zea mays*), complemented by metabolomic and phenotypic analyses. Unlike monomeric human and protozoal ADKs, maize and moss enzymes formed dimers at higher concentrations. Structural and kinetic studies revealed an inactive dimer with active sites blocked by the other subunit. The moss ADKs, with a higher dimerization propensity, showed tenfold lower activity than maize ADKs. Monomeric structures in a ternary complex captured the open-to-closed state transition upon substrate binding. Our findings suggest that oligomerization modulates plant ADK activity, with dimerization serving as a negative feedback mechanism to regulate adenosine and AMP levels [4]. The regulation is even more complex as the plant ADKs form heterocomplexes with SNF1/SnRK1/AMPK [5]. ADK helps to maintain SnRK1 activity which turns off energy-consuming biosynthetic pathways as well as turns on ATP-generating reactions upon various stresses [5, 6].

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We acknowledge SOLEIL for providing synchrotron radiation facilities in using PROXIMA 1 and 2 beamlines (proposals ID 20170872, 20191181, and 20210831). We would like to thank Mgr. Anna Krnáčová for HPLC-MS analysis and Pharm.Dr. Jitka Široká, Ph.D. for measurement of HRMS data. We acknowledge CF Biomolecular Interactions and Crystallography of CIISB, Instruct-CZ Centre, for the assistance of AUC measurements, supported by MEYS CR (LM2023042) and European Regional Development Fund-Project "UP CIISB" (No. CZ.02.1.01/ 0.0/ 0.0/ 18_046/0015974). The work was supported by GA21-07661S from the Czech Science Foundation, IGA_PrF_ 2023_012 from Palacký University, and ANR-11-IDEX -0003. This work benefited from the I2BC crystallization platform supported by FRISBI ANR-10-INSB-05-01.

L11

SHEDDING LIGHT ON THE SECRETS OF NanoLuc, ITS MECHANISM, AND ALLOSTERIC BEHAVIOUR

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NanoLuc luciferase, a small enzyme renowned for its exceptionally bright bioluminescence, has found widespread applications in biotechnology and biomedicine since being designed in 2012 [1]. However, the mystery behind NanoLuc's light-emitting reaction, crucial for developing next-generation bioluminescent systems, remained unsolved. In this study, we made significant progress in understanding NanoLuc's mechanism by combining various laboratory and computational techniques, including crystallography, kinetic measurements, molecular docking, and molecular dynamics simulations with enhanced sampling.

One of the most intriguing features of NanoLuc is its small size, consisting of just 171 amino acid residues. This is a stark contrast to luciferases from sea pansy *Renilla reniformis* (311 residues) and firefly *Photinus pyralis* (550 residues). We confirmed that NanoLuc is monomeric in solution but can also crystallize as a homotetramer under certain conditions. We have also identified two substrate binding sites (Fig. 1): the catalytic site inside NanoLuc monomer, and an allosteric binding site on the oligomerization interface of NanoLuc crystals [2]. Moreover, we used adaptive sampling and adaptive steered molecular dynamics (ASMD) simulations to study the ligand behavior with the NanoLuc monomer and dimer. Importantly, we have demonstrated that introducing mutations in the allosteric site can enhance the bioluminescent reaction occurring in the active site.

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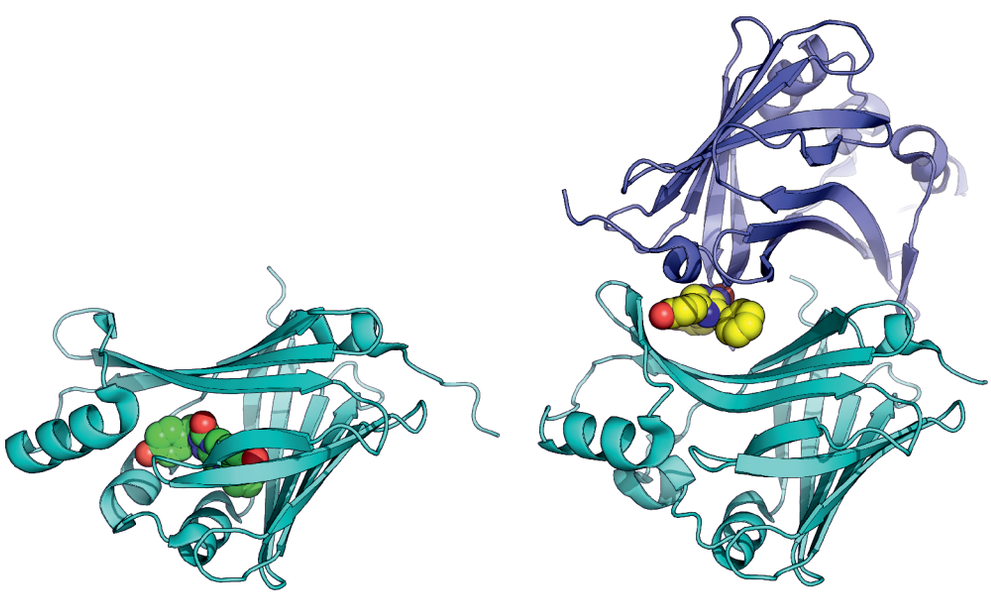

Figure 1. Two distinct substrate binding sites of NanoLuc – the catalytic site (left) and the allosteric site (right).

L12

CDK2-BASED CDK7 MIMIC AS A TOOL FOR STRUCTURAL ANALYSIS: BIOCHEMICAL VALIDATION AND CRYSTAL STRUCTURE WITH SY5609

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Structure-based drug design on CDK7 has not been extensively used due to the lack of a well-established platform for this kinase. CDK7 requires expression in insect cells [1,2], and until recently, there have been only few X-ray structures of CDK7 in the PDB – relatively low-resolution monomeric CDK7 in complex with ATP [1] and with inhibitor LDC4297 (PDB 8p4z). Only very recently, high-resolution cryo-EM structures have been determined for CDK7/cyclin H/MAT 1 complexes with ATP analogue and number of inhibitors including THZ1, samuraciclib and dinaciclib.

In this work, we present CDK2m7 – a CDK2-based CDK7 mimic that can be expressed in *E. coli* in a fully active form. Similar approach has been successfully used for several other kinases, including CDK4 [3]. We identified and mutated 12 CDK residues involved in contacts with ligands to mimic the sequence of CDK7 (Figure 1). CDK2m7 was expressed in *E. coli* in the Thr160-phosphorylated form and co-purified with a fragment of cyclin A2 (cycA, residues 175–432), separately expressed in *E. coli.*

To confirm the suitability of the introduced mutations in CDK2m7, inhibitor binding was analysed using a panel of CDK inhibitors with varying selectivity. Potent CDK7 inhibitors showed no inhibition of CDK2, whereas CDK2m7 was strongly inhibited in nanomolar concentrations. We further determined the crystal structure of active

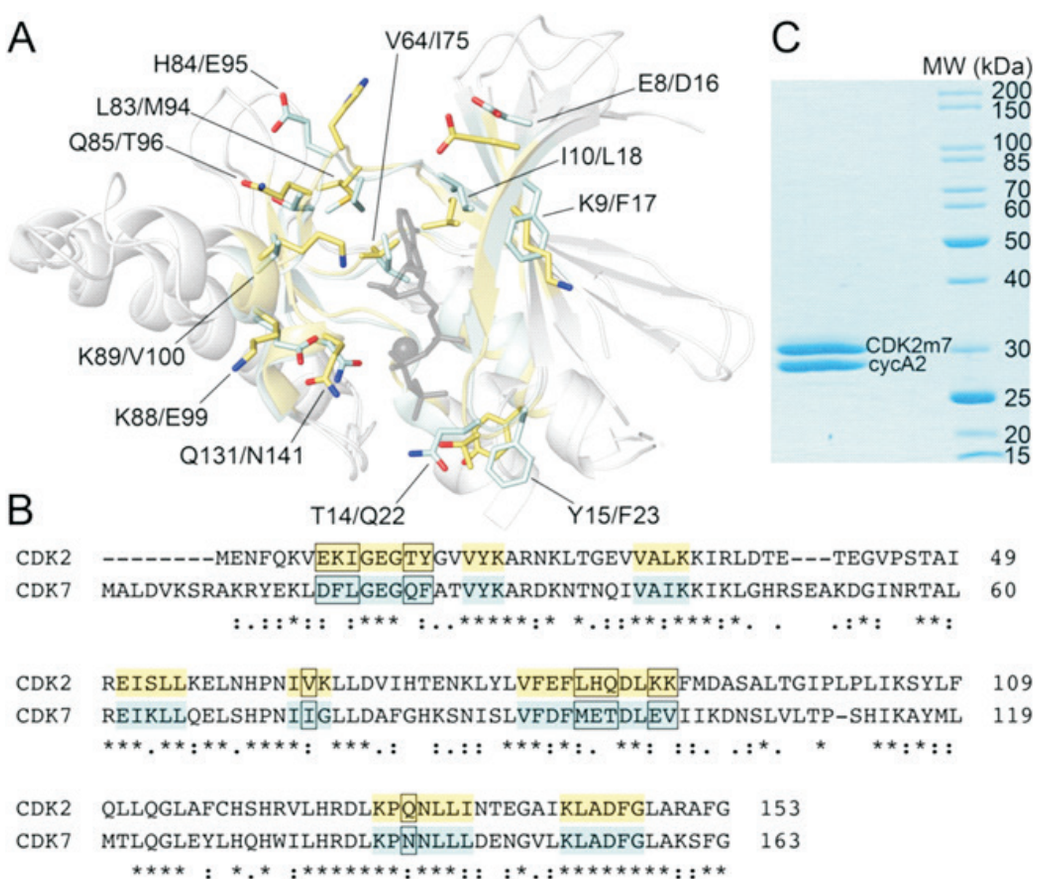

Figure 1. Design of CDK2m7. Structural (A) and sequence (B) alignment of CDK2 (PDB 8fp5) and CDK7 (PDB 1ua2). Regions are shown in yellow and cyan in CDK2 and CDK7, respectively. Mutated residues are shown as sticks (A) and highlighted by boxes (B). (C) Coomassie-stained SDS-PAGE gel documenting the purity of the CDK2m7/cyclin A2 (cycA2) complex.

CDK2m7/cycA in complex with inhibitor SY5609 [4] at the resolution of 2.2 Å. It was clear that CDK2m7 maintains the integrity of the active site, documented by similar conformation of the side-chains of the mutated residues as in the structures of the CDK7/cycH/MAT1. Moreover, binding of the inhibitor corresponded well with the previously performed molecular docking of SY5609 into the active CDK7 complex (PDB 7b5q) [4]. In conclusion, CDK2m7 mimics well the CDK7, could be produced in *E. coli* in a fully active, phosphorylated form in complex with cyclin A2. CDK2m7 could be used in biochemical measurements as well as in structure-assisted design of CDK7 inhibitors.

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We would like to thank Dr. Petr Pachl from IOCB Prague for diffraction data collection and Dr. Martin Lepšík of High Performance Computing Core Facility of IOCB Prague for advice on inhibitor geometry and binding. We acknowledge SOLEIL for provision of synchrotron radiation facilities and we would like to thank Dr. Martin Savko for assistance in using beamline PX2a. The work was supported by project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

L13

CRYSTALLIZING THE FUTURE: UNVEILING NEW INNOVATIONS AT MOLECULAR DIMENSIONS

Paul Driver

Molecular Dimensions

Molecular Dimensions has long been a leader in providing comprehensive workflow solutions that empower structural biologists to overcome challenges in protein crystallization. In this presentation, the speaker will offer an in-depth update on the company's latest commercial innovations. First, a novel ultrafiltration concentrator will be introduced—a breakthrough product that eliminates the need for a centrifuge, delivers gentle concentration of recombinant proteins, and achieves exceptionally high recovery rates. In addition, the talk will spotlight a new lipid screen developed to evaluate the optimal lipids for membrane protein workflows, a vital step in enhancing the stability and function of these challenging targets. Furthermore, an updated suite of crystallisation screens will be presented, each designed to streamline the process of protein structure determination. By detailing these advancements, the presentation underscores Molecular Dimensions' commitment to innovation and its ongoing role in driving the evolution of structural biology research.

Friday, March 21, Session IV

L14

STRUCTURAL MECHANISMS OF REGULATION OF HUMAN Nedd4-2 BY 14-3-3η DIMER AND CALCIUM IONS

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Nedd4-2 (NEuronal precursor cell-expressed Developmentally Down-regulated 4-2) ubiquitin ligase is a member of the Nedd4 HECT E3 family with whom it shares the same domain architecture: C2 domain (membrane- and calcium-binding), four WW domains (substrate recognition) and bilobed HECT domain (catalytic activity). It is the last enzyme of the ubiquitination cascade, responsible for altering the function and activity of its targets by attaching variable ubiquitin chains to them. Nedd4-2 is involved in numerous signalling pathways and its dysfunction is linked to different pathophysiological conditions (Liddle syndrome – form of hypertension, respiratory distress, heart and kidney diseases, epilepsy and so on), which highlights the importance of understanding its regulation. Proposed mechanisms so far include autoinhibition caused by intramolecular binding [1], activation by calcium ions [2] and intermolecular interactions [3,4].

We described how 14-3-3η homodimer (binds to pSer residues surrounding the WW2 domain) and calcium ions affect Nedd4-2 using following methods: liposome-binding and ubiquitination assays, analytical ultracentrifugation, X-ray Crystallography, SAXS, H/D exchange coupled to Mass Spectrometry and CryoEM. Our results show that calcium is necessary for membrane binding but not for activating Nedd4-2. We found which amino acids interact with these ions and that they don't cause significant structural reorganization in contrast to membrane binding. Our CryoEM model described the specific way Nedd4-2 domains interact to keep it inactive. The complex of Nedd4-2:14-3-3η wasn't influenced by calcium but was inhibitory of the enzyme's ability to bind to membranes and perform ubiquitination.

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This study was supported by the Czech Science Foundation (V.O., grant number: 23-04686S), the Czech Academy of Sciences (RVO: 67985823 of the Institute of Physiology) and the Visegrad Scholarship (ID number: 52310440).

L15

SERVALCAT AND *METALCOORD*: NEW STRUCTURE REFINEMENT STRATEGIES IN *CCP4* AND *CCP-EM*

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In recent years, the field of structural biology has seen rapid advancements, which in turn has driven the scientific software development. Here, we present new features for atomic model refinement within the *CCP4* [1] and the *CCP-EM* [2] software suites.

Servalcat [3] is a comprehensive atomic model refinement program drawing inspiration from *Refmac5*/ *Refmacat* [4] software tools. Recently, it has been integrated into the *CCP4i2* and *CCP-EM Doppio* graphical user interfaces making it accessible to users.

For crystal structures, *Servalcat* allows direct refinement against reflection intensities, eliminating the need for French-Wilson conversion to structure factor amplitudes. This approach enhances the quality of the resulting density maps, such as producing more detailed omit maps for partially occupied ligands. Additionally, the program is also now able to do refinement against twinned data addressing some of the limitations previously encountered in the *Refmac5* program.

Moreover, for cryoEM structures, *Servalcat* offers several advanced features, such as refinement under point group or helical symmetry constraints, a weighted and sharpened $F_o - F_c$ difference map for validation, and half-map cross-validation.

MetalCoord [5] is a program designed to tackle one of the longstanding problems that is the modelling and refinement of metal coordination environments in macromolecular structures (Figure 1). It performs a thorough analysis of metal-coordination geometries based on reference data extracted from the Crystallography Open Database (COD) [6]. As a result, ideal stereochemical information is provided, given as external distance and angle restraints, which can then be used in subsequent structure refinement by *Servalcat*. *MetalCoord* is currently available from GitHub (*https://github.com/Lekaveh/MetalCoordAnalysis*) and is planned for inclusion in the *CCP4* Suite.

The *MetalCoord* program has also been incorporated within the *AceDRG* program [7] to provide improved stereochemical dictionaries for metal-containing monomers. The enhanced tools have been used to update all metal containing ligands available from the latest version

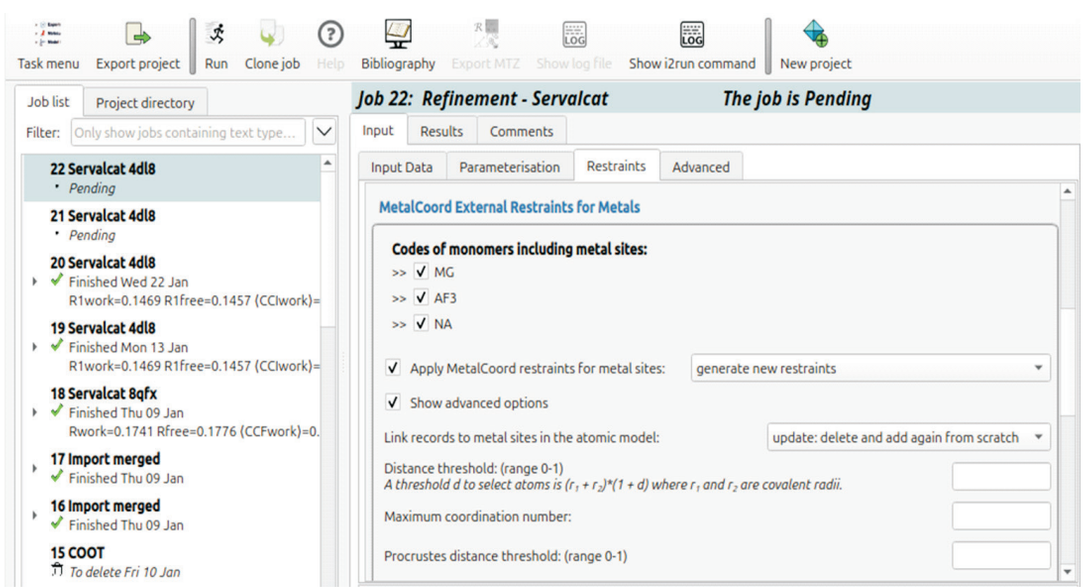

Figure 1. Implementation of *MetalCoord* [5] restraint generation within the *Servalcat* [3] structure refinement pipeline in *CCP4i2* [1].

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L16

FROM CHARACTERIZATION OF PROTEINS AND THEIR INTERACTIONS TO THEIR DETERMINATION IN NANOCARRIERS

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The study of proteins using complementary analytical techniques is crucial for successful basic research or its application in many areas of science. ANAMET s.r.o. offers leading analytical instrumentation from basic protein characterization, and determining their interactions, to determining protein presence in extracellular vesicles or other nanocarriers.

Differential scanning calorimetry (DSC) is capable of monitoring heat changes associated with protein structural transitions, determining the melting temperature and thereby studying the effects of mutations, chemical modifications or ligand binding on protein stability. Gel permeation chromatography (GPC) is essential for determining the molecular weight and size distribution of proteins. It is particularly useful for assessing the purity of protein samples by detecting oligomerization or aggregation states. Isothermal titration calorimetry (ITC) monitors the thermodynamics of protein-ligand interactions to quantify binding affinity, kinetics, stoichiometry, and thermodynamic parameters such as enthalpy and entropy. ITC provides real-time, label-free data on interactions in solution and offers a direct understanding of how proteins interact with ligands such as small molecules, DNA or partner proteins. Grating-coupled interferometry (GCI) is a highly advanced on-chip technique for assessing molecular interactions technologically close to SPR. By measuring phase shift in the optical guided wave by the chip surface caused by the binding between analyte and ligand, high sensitivity is achieved even for very weak bonds whose values could not be measured before. GCI provides information on binding kinetics, equilibrium dissociation constant (KD) and association and dissociation rates. Flow cytometry in nanoliter volumes nanoFCM is the only analyzer of its type developed specifically for nanoparticle analysis and you are able to determine the presence of proteins on the surface of extracellular vesicles, determine the protein content of liposomes, or determine protein expression by viral particles. In addition to detecting fluorescent labels, you are also able to determine the size and concentration of particles in the sample.

ANAMET s.r.o. offers all the above mentioned techniques in collaboration with Malvern Panalytical and nanoFCM and we are able to provide you with complete service and application support. If you are interested in these techniques I would be happy if you could look me up during the conference or contact me at macha@anamet.cz.

Friday, March 21, Session V

L17

AMINO ACIDS IN THE CONTEXT OF PROTEIN STRUCTURE: WHAT WE CAN LEARN FROM PROTEIN LARGE LANGUAGE MODELS

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Proteins are extremely diverse in structures and functions, despite the fact that they are composed only from 20 amino acids. Any of these amino acids can play a different structural and functional role depending on the context of the sequence and structure. For example, histidine can be a charged residue on the protein surface, a specialized residue in "catalytic triad", a metal-chelating residue, etc. Analysis of this context was recently enabled by advanced machine learning methods originally developed for the analysis of natural language. It is possible to analyze the amino acid sequence of a protein as a "sentence" composed of amino acid residues as "words". We analyzed sequences of all human proteins using a large language model ESM-2 (Evolutionary Scale Modeling). Each amino acid residue was converted into its profile that reflects its structural role in the context of the overall sequence and structure of the protein. These profiles can be visualized as a 2D map. We plan to annotate this map to assign a 3D structure to each cluster of this map. We will illustrate how this map can help us to understand the structure and function of different regions of any protein.

The work was supported by COST (ML4NGP, CA21160, LUC 24136).

L18

GATING OF THE RIBOSOME EXIT TUNNEL

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Ribosomes are complex biomolecular machines essential for proteosynthesis and thus for life as we know it. As the nascent peptide is assembled one amino acid residue at a time, the growing chain escapes through the ribosomal tunnel. The ribosomal tunnel is mostly made up of rRNA. However, there is a narrowing of the tunnel, called the constriction site (CS), formed by the extended loops of two ribosomal proteins: uL4 and uL22. This CS is where the first protein--protein contact of the nascent chain takes place. The tips of uL4 and uL22 include charged amino acid residues that come into close contact with the nascent chain and may be of special importance in the early stages of translation.

To understand the conformational variability of the CS in various chemical contexts, we explored 222 experimental *E. coli* ribosome structures from the Protein Data Bank

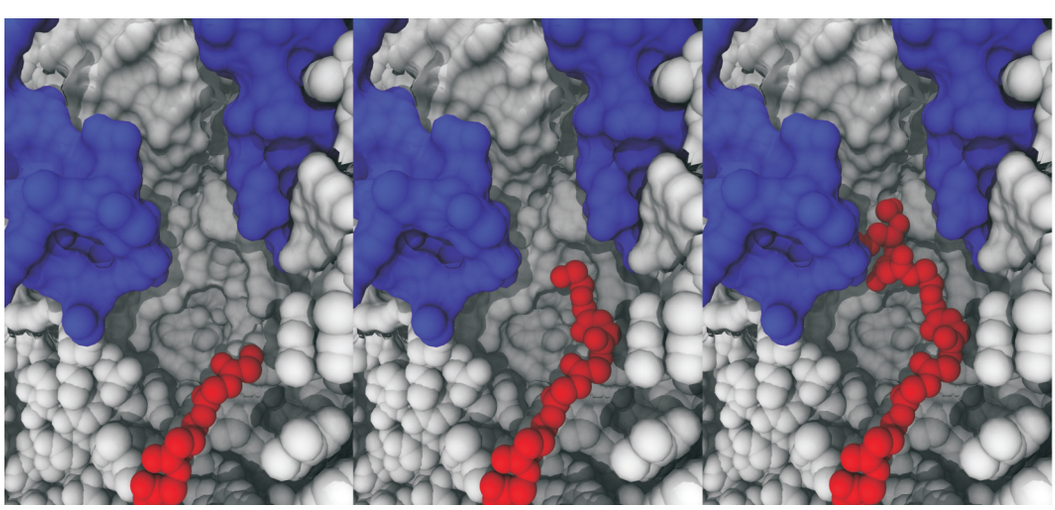

Figure 1. Varying lengths of polyglycine (red) in proximity to the constriction site (blue) in the ribosomal tunnel.

and their distribution of distances of the tips of uL4 and uL22. These ribosomes contained various cofactors, mutations, or ligands. Our analysis reveals that the CS is flexible and the uL4 and uL22 tip distance -- hence the tunnel width -- varies in different chemical contexts.

In our ongoing research, we are exploring the CS dynamics and the interaction of a nascent peptide with the CS using molecular dynamics simulations. We study four systems with varying lengths of polyglycine (11, 8 or 5 glycines or no peptide at all) in the ribosomal tunnel. In the talk, I will present our preliminary results that suggest that the peptides affect not only the dynamics of the CS but also more distant parts of the ribosome.

L19

LONG-RANGE ELECTRON TRANSFER IN PROTEIN-METAL JUNCTIONS

G. N. Jonnalagadda[1], L. Hronek[1], X. Wu[2], and Z. Futera[1]

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Cytochrome b_{562} (Cyt b_{562}) is a small redox-active heme protein that has served as a key model system for understanding biological electron transfer processes. Electron transport in such proteins plays a crucial role in various biochemical functions, including respiration and enzymatic catalysis. Investigating its transport properties in protein-metal junctions provides valuable insights into charge transfer mechanisms relevant to bio-electronic interfaces. Recent experimental studies have demonstrated the conductive properties of Cyt b_{562} on gold surfaces [1], but a deeper theoretical understanding of its charge transport mechanism is necessary. This study presents a comprehensive theoretical analysis of electron transport in Cyt b_{562}-based junctions using a multiscale computational approach, examining both coherent and incoherent transport processes.

To model electron transport, molecular dynamics (MD) simulations were employed to generate junction geometries under both vacuum-dried and solvated conditions, where the protein was covalently bound to gold contacts in various configurations [2]. Charge transport was analyzed through two mechanisms: coherent tunneling, studied using the Landauer-Büttiker formalism within the Density Functional Theory (DFT) framework, and incoherent hopping, modeled using the semi-classical Marcus theory.[3]

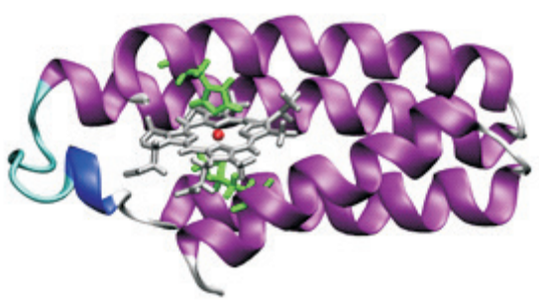

Figure 1: Crystal structure of Cyt b_{562}

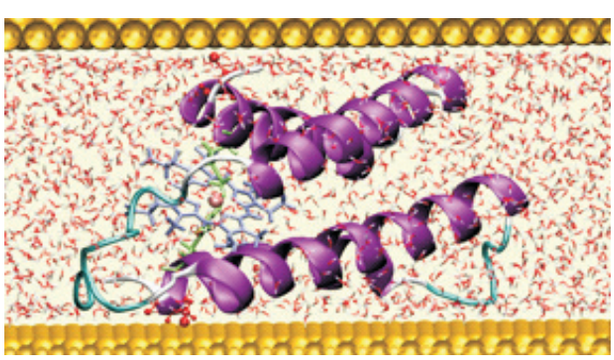

Figure 2: Solvated lying junction of Cyt b_{562}

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L20

HIGH-RESOLUTION LIQUID CELLS FOR TIME-RESOLVED CRYO-EM

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A large portion of protein dynamics occurs on micro- and millisecond time scales, including large-scale domain motions and protein folding processes [1]. Insight into the dynamic states of these processes is necessary for understanding how proteins function.

Microsecond time-resolved cryo-electron microscopy (Cryo-EM) has recently emerged as a promising method for capturing intermediate states reached after tens of microseconds of dynamics [2, 3]. A cryo specimen is locally flash melted using a laser beam *in situ* of a transmission electron microscope. As the laser is switched off, the specimen revitrifies, trapping the particles in their transient states. The time window for the dynamics (time-in-liquid) is tuned by the duration of the laser pulse and usually sits at 30 µs with the temporal resolution of the method is better than 5 µs [4]. Moreover, the near-atomic resolution capabilities of standard single-particle Cryo-EM are preserved even after the flash melting process [5]. However, exceeding time-in-liquid beyond 30 µs has proven to be difficult using this setup.

The inherent limitation of the above-described method stems from evaporation of the sample in the liquid phase. This limits the maximum achievable time-in-liquid to the tens of microseconds, as well as the maximum reachable temperature, which plateaus at approximately room temperature due to evaporative cooling [4, 6].

To overcome this limitation, we developed a method that prevents evaporation by depositing a sealing membrane onto the cryo sample prior to laser flash melting. This is achieved using physical vapor deposition of silicon dioxide in an in-house built setup. The thickness of the deposited sealing layer is precisely controlled and can be as thin as 1.5 nm on each side of the sample. This thickness is sufficient to retain the sample even after 300 µs spent in the liquid, extending the observational time-window for protein dynamics from tens to hundreds of microseconds and. Moreover, due to the absence of evaporative cooling of the specimen, temperature jump experiments can also be performed. Importantly, the resolution loss resulting from the sealing layers is negligible, as demonstrated by sub-1.8 A resolution reconstruction of apoferritin.

Experiments prove that time windows up to milliseconds can be achieved with a sufficiently thick membrane (e.g., 10 nm on each side), opening new possibilities not only in time-resolved Cryo-EM, but also in liquid cell electron microscopy.

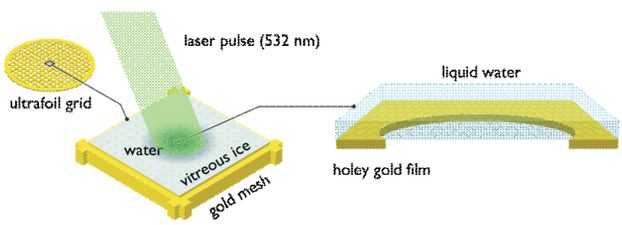

Figure 1. Schematic principle of laser melting. A cryo sample on a gold grid is first illuminated by a laser beam, causing heating of the gold film and subsequent local melting of the vitreous ice. Surrounding areas on the grid square, as well as remaining grid squares, remain unaffected by melting.

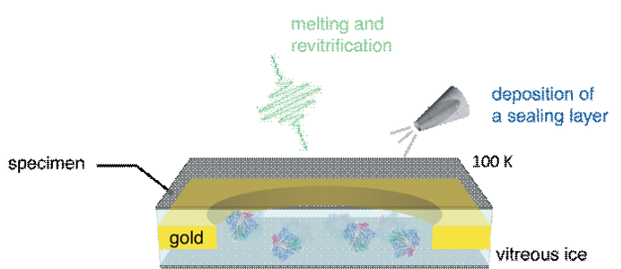

Figure 2. Sealing and revitrification of a cryo sample. First, silicon dioxide membranes are deposited from both sides onto the vitreous ice. Subsequently, sample is flash melted with a microsecond laser pulse, allowing for the dynamics to occur. After the laser is switched off, specimen revitrifies and traps the particles in their transient state. Thanks to the SiO2 membranes, the specimen evaporation is limited.

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L21

STRUCTURAL BASIS OF THE 14-3-3/CYCLIN Y-MEDIATED REGULATION OF CDK16

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CDK16, also known as PCTAIRE1, is a member of the PCTAIRE family of cyclin-dependent kinases (CDKs). CDK16 plays a crucial role in various physiological processes, such as neurite outgrowth, vesicle trafficking, spermatogenesis, glucose homeostasis, and muscle differen-t iation. [1] Unlike other CDKs, CDK16 activation is mediated through interaction with cyclin Y, phosphorylated at residues Ser100 and Ser326, in complex with 14-3-3, as depicted in Figure 1. CDK16 has a distinct unstructured N-terminal extension that includes two residues, Ser119 and S153, these residues can undergo PKA phosphorylation, resulting in the formation of 14-3-3 consensus binding motifs. When phosphorylated, these residues are reported to decrease CDK16 activity [2]. CDK16's activity is associated with the progression of various cancers, including breast cancer, lung cancer, endometrial cancer, melanoma, and others. In this context, the phosphorylation of specific substrates promotes cell proliferation [3]. Research has demonstrated that inhibiting CDK16 activity leads to a reduction in cancer cell growth [4]. Given CDK16's crucial role in regulating cell proliferation in cancer, it is essential to understand its activation mechanism at the molecular level. Herein, we solved the structure of the complexes involved in CDK16 activation using Cryo-EM, showing the role of cyclin Y and 14-3-3 in CDK16 activation.

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This work was supported by Czech Science Foundation Grant No. 25-15222S.

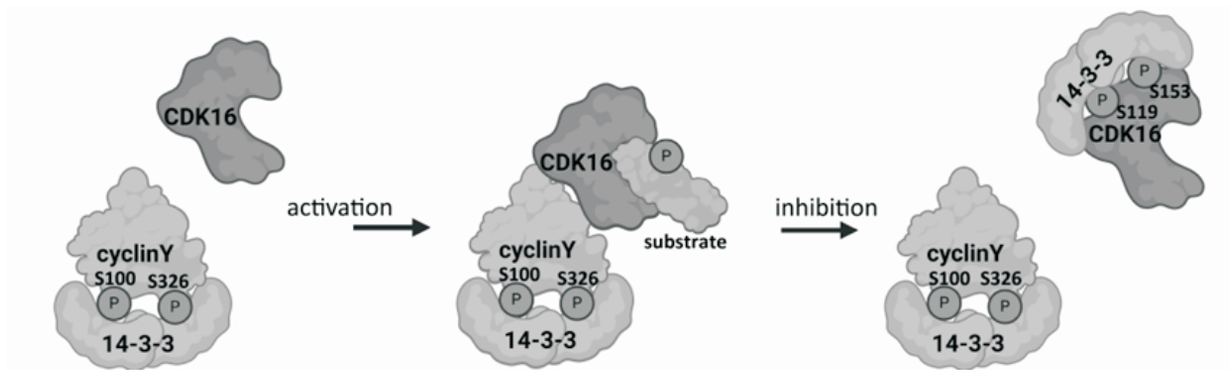

Figure 1. Graphical scheme of CDK16 regulation via cyclin Y and 14-3-3. Created with BioRender.com

L22

SEEING IS BELIEVING: PROXIMITY LIGATION ASSAY

Stanislav Kukla

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The Duolink proximity ligation assay (PLA), an exclusive product of Merck, represents a powerful technique for studying protein interactions in situ at endogenous protein levels, enabling researchers to visualize and quantify interactions at the cellular level. This simple yet innovative method employs a unique combination of proximity ligation and fluorescence microscopy, providing high sensitivity and specificity in detecting and localizing biomolecular interactions. The assay utilizes two primary antibodies that bind to the target proteins, which are then linked by DNA oligonucleotides. When the antibodies are in close proximity, a ligation reaction followed by DNA amplification with fluorescent probes occurs, generating a detectable signal.

The benefits of Duolink PLA include its ability to provide spatial information about protein interactions within their native cellular context, facilitating the study of complex biological processes. Furthermore, the assay's compatibility with various sample types and its potential for multiplexing allow for comprehensive analyses of protein networks. Merck offers full application support to ensure optimal use of the Duolink PLA, assisting researchers in maximizing their experimental outcomes. This presentation will delve into the technical aspects of the Duolink PLA, highlight its advantages over traditional methods, and discuss its typical applications in the field of life sciences.

Duolink PLA web page

www.sigmaaldrich.com/duolink

Antibody explorer web page

www.sigmaaldrich.com/antibodies

Saturday, March 22, Session VI

L23

CRYO-EM ANALYSIS OF *E. coli* RIBOSOME RECOVERY MECHANISM IN THE ABSENCE OF THE 30S MATURATION FACTOR RimM

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Ribosome biogenesis is a complex, multistep process that involves the folding and modification of ribosomal RNA (rRNA), followed by the sequential assembly and integration of ribosomal proteins [1]. This intricate process is tightly regulated by numerous biogenesis factors that ensure the proper formation of functional ribosomal subunits [1]. Disruptions in these factors can lead to severe growth defects and the accumulation of immature ribosomal subunits, highlighting their critical role in ribosome maturation [1].

RimM, a key ribosome maturation factor, facilitates the correct assembly of the 30S small ribosomal subunit [2]. In *E. coli*, deletion of the *rimM* gene results in impaired growth, accumulation of immature 30S particles, and reduced translational efficiency [3-4]. Interestingly, the gradual recovery of bacterial growth suggests the presence of compensatory mechanisms that restore translation capacity over time. One such mechanism may involve the ribosomal silencing factor RsfS, which regulates protein synthesis by binding to the 50S ribosomal subunit [5]. This interaction prevents premature 70S ribosome formation, potentially shielding mature 50S subunits from associating with immature 30S particles.

In this study, we employed single particle cryo-electron microscopy (cryo-EM) to investigate the interplay between RsfS and translation initiation factors in the absence of RimM. Our structural analysis reveals that translation initiation factors bind to immature 30S subunits, preventing their association with 50S subunits until ribosomal proteins are fully assembled on the 30S subunit. Concurrently, RsfS binds to the 50S subunit, effectively inhibiting the formation of 70S ribosomes. These findings provide valuable structural and mechanistic insights into the final stages of ribosome assembly and underscore the critical roles of ribosome-associated factors in maintaining translational fidelity and cellular adaptation.

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This study was supported by the LL2008 project with financial support from MEYS CR as a part of the ERC CZ program (G.D.).

L24

SNAPSHOTS OF THE GLUCOSE METABOLISM STUDIED BY ELECTRON CRYO-MICROSCOPY *IN VITRO* AND *IN SITU*

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Insulin is a key hormone responsible for maintaining glucose homeostasis. It is stored in pancreatic cells in a form of dense granules. We have used FIB/SEM microscopy and correlative light-electron microscopy (CLEM) to quantify the granule presence in different cell lines and studied their structure. Insulin receptor (IR) is a receptor tyrosine kinase which upon insulin binding on the extracellular receptor domain induces autophoshorylation reaction on its cytoplasmic domains. Misregulation in the insulin signalling is a cause of Diabetes melitus I and II. We have studied the mechanism of IR inhibition with insulin non-related peptidomimetics capable to fully antagonise insulin action to prevent aberrant IR signalling.

L25

CLINCELIN: A REDESIGNED LINCOSAMIDE COMBATS RIBOSOME RESISTANCE MODIFICATION THROUGH ENHANCED BINDING AND STRUCTURAL FLEXIBILITY

Michaela Novotná[1,2], Vladimir Vimberg[1], Fanny Boissier[3], Markéta Koběrská[1], Barbora Kolrosová[1], Julie Pokorná[1], Marie Brajerová[5], Radek Gažák[4], Petr Slavík[6], Lada Hanzlíková[4], Kryštof Šigut[6], Marcela Krůtová[5], Zdeněk Kameník[4], C. Axel Innis[3], Jiří Janata[4], Gabriela Balíková Novotná[1]

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Lincosamides, an important class of antibiotics in human medicine, inhibit translation by binding to the catalytic centre of the ribosome. However, their efficacy is impaired if the ribosome bears widespread A2058 methylation conferred by the *erm* resistance gene, rendering all clinical lincosamides ineffective. In this study, we present clincelin, a novel chimeric compound structurally derived from the natural lincosamides celesticetin and lincomycin [1]. Rigorous *in vitro* tests show that clincelin has significantly higher antibacterial activity compared to the two parent compounds and the clinically used lincosamide clindamycin. Remarkably, clincelin retains its efficacy also against *erm*-mediated resistant strains. Cryo-EM analysis reveals the unique mechanism underlying clincelin's evasion of resistance: Not only does it exhibit improved binding contacts with the ribosome, but it also has remarkable structural flexibility that allows different binding modes depending on the presence of Erm methylation. To the best of our knowledge, clincelin is the first antibiotic to exhibit such anti-resistance adaptation to overcome the resistance. Our detailed characterization paves the way for the development of next-generation lincosamides for clinical use and establishes a paradigm for overcoming antibiotic resistance through molecular design.

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This work was supported by the Technology Agency of the Czech Republic within the TREND Programme (Project No. FW03010628); project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU; International Mobility of Researchers of the Institute of Microbiology of the CAS, v.v.i. No 2, (Project No. CZ.02.2.69/0.0/0.0/18_053/0017705); Agence Nationale de la Recherche (ANR) (Grant No. ANR-22-CE11-0012 RibioTiX); and Grant Agency of Charles University (Grant No. 134324).

L26

STRUCTURE OF BOTULINUM-LIKE TOXINS

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[2]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Czech Republic
jana.skerlova@uochb.cas.cz

Botulinum neurotoxins (BoNTs), produced by *Clostridium botulinum*, are the most potent toxins known to man and are used to treat an increasing number of medical conditions [1]. They target neuromuscular junctions and inhibit synaptic vesicle exocytosis in motor neurons, thereby causing flaccid paralysis. Recently, a new BoNT serotype (BoNT/X) was identified in a botulism patient and *bont* genes were discovered in bacteria outside the *Clostridium* genus [2], such as *Weissela oryzae* (BoNT/Wo) or *Enterococcus faecium* (BoNT/En). These botulinum-like toxins share the molecular architecture of BoNTs and are accompanied by the non-toxic non-hemagglutinin protein (NTNH), which forms the minimal progenitor toxin complex with the toxin to protect it in the host digestive tract.

We used single-particle cryo-EM to determine the structures of the minimal progenitor toxin complexes of

botulinum-like toxins BoNT/X [3] (Figure 1) and BoNT/Wo [4]. The structures demonstrate how the toxins interact with their non-toxic protective partners and help us understand the evolutionary relationships between BoNT-like proteins. Moreover, they provide a platform for the engineering of new scientific tools and potentially also novel therapeutic toxins.

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This work was supported by grants from the Swedish Research Council and the Swedish Cancer Society to P.S. The work of J.Š. was supported from ERDF/ESF project "UOCHB Mobility" (No. CZ.02.2.69/0.0/0.0/16_027/0008477) granted to the IOCB of the CAS. Allocation of cryoEM instrument time by the Cryo-EM Swedish National Facility is gratefully acknowledged and the CIISB research infrastructure project LM2018127 funded by MEYS CR is gratefully acknowledged for the financial support of the measurements at the CF CryoEM.

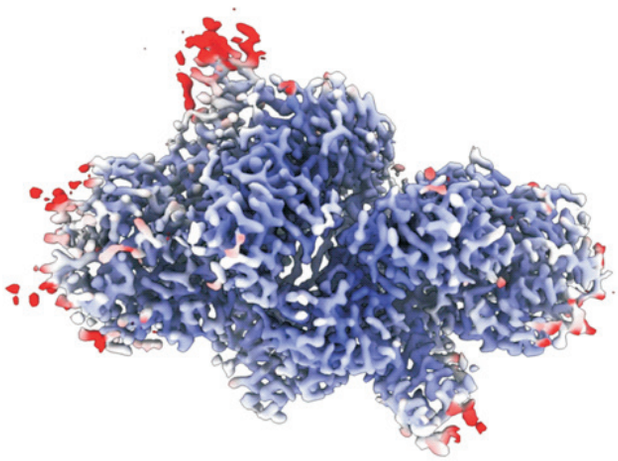

Figure 1. Cryo-EM reconstruction of the minimal progenitor toxin complex of BoNT/X.

Saturday, March 22, Session VII

L27

IN SITU VISUALIZATION OF THE *BORDETELLA* FILAMENTOUS HEMAGGLUTININ BY CRYO-ELECTRON TOMOGRAPHY

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Bordetella pertussis, the causative agent of whooping cough, relies on the surface adhesin filamentous hemagglutinin (FhaB) for adherence and colonization of respiratory tract cells. FhaB is initially translated as a 360-kDa precursor and later released into the external environment as a 'mature' 220-kDa protein; however, its structural properties remain poorly understood. Here, we describe two approaches for the preparation and characterization of bacterial minicells derived from *B. pertussis* and *B. bronchiseptica*. These minicells were generated by deleting the *minD* gene and introducing the A126V substitution in the *mreB* gene in the bacterial chromosome, as well as by overexpressing *FtsZQ* genes under the control of the *pBAD* promoter. Minicells were collected from cultures of the mutant *Bordetella* strains using differential and gradient centrifugation. Purified minicells were used for in situ visualization of the FhaB molecule via cryo-electron tomography. Tomograms revealed that the FhaB molecule forms a ~35-nm long filament protruding from the minicells surface. These findings highlight the potential of minicells as a powerful tool for structural investigations of *Bordetella* virulence factors *in situ*.

L28

TWO SOLUTIONS FOR EFFICIENT LIGHT-HARVESTING IN PHOTOTROPHIC *GEMMATIMONA*DOTA

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The anoxygenic phototrophic bacterium *Gemmatimonas phototrophica* prefers growth at lower-light intensities. It has evolved a unique type of photosynthetic complex that consists of a type-2 reaction centre surrounded by two rings of light harvesting antenna with each giving rise to a distinct near infrared absorbance band. A closely related species *Gemmatimonas groenlandica* grows better at higher-light intensities and contains the same photosynthesis gene cluster, yet its photosynthetic complex has a notably different near infrared absorption spectrum with only one large absorption band. In order to understand the origin of this difference, the structure of the photosynthetic complex from this species was determined by the cryogenic electron microscopy. The analysis revealed that it also contains two rings but that the outer antenna ring absorption is red-shifted. The shift was caused by rotation of a tryptophan residue side chain to form a H-bond with bacteriochlorophyll and increased the strength of the intra-dimer exciton coupling. In addition, the outer antenna ring lacks monomeric bacteriochlorophylls. This loss reduced the optical antenna cross-section in *Gemmatimonas groenlandica*, but the H-bond increased the probability of exciton exchange among the complexes (connectivity). Therefore, these evolutionary changes have changed the higher-light optimised complex present in *Gemmatimonas groenlandica* into a complex that has allowed *Gemmatimonas phototrophica* to grow well under lower-light intensities.

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L29

CELL ENTRY AND GENOME DELIVERY OF ENTEROVIRUSES

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Enteroviruses enter cells by receptor-mediated endocytosis. However, it is not fully understood how enteroviruses release their genomes and how enterovirus particles or RNA genomes cross the endosome membrane into the cytoplasm. We used cryo-electron microscopy to visualize enterovirus particles in the process of genome release. The exit of the RNA results in a loss of one, two, or three adjacent capsid-protein pentamers from a particle. The opening in the capsid, which is more than 120 Å in diameter, enables the release of the genome without the need to unwind its putative double-stranded RNA segments. We used cryo-electron tomography of infected cells to show that endosomes containing enteroviruses deform, rupture, and release virus particles into the cytoplasm. Blocking endosome acidification with bafilomycin A1 reduced the number of particles that released their genomes but did not prevent them from reaching the cytoplasm. Inhibiting post-endocytic membrane remodeling with wiskostatin promoted abortive enterovirus genome release in endosomes. Our results show that cellular membrane remodeling disrupts enterovirus-containing endosomes and thus releases the virus particles into the cytoplasm to initiate infection. The cells also contained empty capsids lacking pentamers of capsid proteins. Since the studied enteroviruses employ different receptors for cell entry but are delivered into the cytoplasm by cell-mediated endosome disruption, it is likely that most, if not all enteroviruses, and probably numerous other viruses from the family *Picornaviridae*, can utilize capsid opening and endosome rupture to infect cells.

Posters

P1

PROTEIN PRODUCTION FACILITY – DNA & PROTEINS FOR YOUR RESEARCH

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The Protein Production core facility at the Centre of Molecular Structure (CMS) offers comprehensive services, covering every step from DNA to the purified protein. These include gene cloning into expression vectors, site-directed mutagenesis, and protein expression followed by protein purification.

Our cloning services include both traditional cloning using restriction enzymes and restriction free (RF) methodologies. Customers can provide us with their templates, or we can order them from external companies. Subsequently, we can deliver and test several of our plasmids. Furthermore, we perform small-scale expression and solubility tests using various *Escherichia coli* strains under different conditions. We are expanding our services by introducing eukaryotic production. We can provide protein production in human embryonic kidney cells (HEK) or baculoviral expression system using Sf9 insect cells as an alternative to prokaryotic expression. Finally, we offer large-scale production and purification of target proteins.

In protein purification, we employ a range of steps, such as affinity chromatography (GST-tag purification, Strep-Tactin XT Sepharose or immobilized metal chelate affinity chromatography), performed either on an FPLC or in gravity flow setups. We also offer an ion-exchange chromatography, and size exclusion chromatography using Superdex columns (75 or 200, 10/300 increase, or HiLoad 16/600). Customers can request modifications to standardized protocols or provide us with established protocols for implementation.

In 2025, our team aims to organize the first workshop, expand and welcome new colleagues, customers, and collaborations.

The Biocev Protein Production core facility is a part of CMS operated by the Institute of Biotechnology, Czech Academy of Sciences as a member of the Czech Infrastructure for Integrative Structural Biology (ciisb.org). 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 by European Regional Development Fund-Project „Innovation of Czech Infrastructure for Integrative Structural Biology“ (No. CZ.02.01.01/00/23_015/0008175).

Figure 1. Laboratory and services of the CF Protein Production at CMS.

P2

THE EFFECT OF THE CARDIAC-ASSOCIATED MUTATIONS ON THE BIOPHYSICAL PROPERTIES, FOLD AND STRUCTURE OF THE N-TERMINAL DOMAIN OF HUMAN RYANODINE RECEPTOR 2

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Calcium ions play a key role in physiological processes, such as the excitation-contraction process in muscle cells. The regulation in human cardiac muscle cells is primarily mediated by the human ryanodine receptor 2 (hRyR2). hRyR2 is a large calcium channel that mediates the transfer of calcium cations from the sarcoplasmic reticulum to the cytosol of cardiomyocytes. hRyR2 consists of four monomers, each composed of 10 domains. One of these domains is the N-terminal domain (NTD), where approximately 70 point mutations associated with severe arrhythmias have been identified. Mutations in the NTD cause several cardiologic disorders, primarily catecholaminergic polymorphic ventricular tachycardia 1 (CPVT1) and arrhythmogenic right ventricular dysplasia 2 (ARVC/D2).

In our study, we focused on the biophysical characterization and of hRyR2 NTD in its wild-type and mutant forms. We successfully expressed and purified wild-type and M81L and L433P mutant fragments of the hRyR2 NTD. We also verified their fold by CD spectroscopy, determined the influence of the mutation on the hRyR2 NTD size by FIDA analysis and determined their thermal stability using nanoDSF. We found that the M81L mutation increases the thermal stability of hRyR2 NTD. This mutant was also successfully crystallized. The L433P mutation reduces the solubility of hRyR2 NTD, affects its oligomeric state and alters the structure of this domain.

Authors thank to Gabriel Žoldák and Michal Nemergut from CIB UPJŠ Košice, Slovakia for CD-spectra measurements and Jozef Uskoba, BioTech Czechia for FIDA measurements. The study was financially supported by grant VEGA 2/0081/24.

P3

RIBOTUNER – THE INTERPLAY OF ANTIBIOTICS, RIBOSOMES AND ABCF ATPASES IN FINE TUNING GENE EXPRESSION

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Antibiotics that bind to the catalytic centre of the bacterial ribosome interact directly with the nascent peptide, leading to variability in the inhibitory effect that depends on the specific amino acid sequence of the nascent peptide. However, this phenomenon, known as context-dependent translation inhibition, has not yet been studied with classes of antibiotics that inhibit translation initiation, such as lincosamides, pleuromutilins and streptogramins A (LSaP). Bacteria utilise the context-dependent inhibition of translation by ribosome-targeting antibiotics to modulate gene expression: Ribosome which is blocked only by specific antibiotic while translating regulatory uORFs in the 5'UTR of target mRNAs triggers conformational changes that allow transcription or translation. This mechanism, known as ribosome-mediated attenuation, plays a critical role in the control of antibiotic resistance genes encoding ABCF ATPases, which also regulate gene expression in response to antibiotics. Our results suggest that context dependency and ribosome-mediated attenuation are important for ABCF-mediated antibiotic signalling. Using gene reporter assays and in vitro ribosome profiling techniques, we decipher the context dependency of LSaP antibiotics, their effects on the regulation of ABCF protein-encoding genes and how ABCF protein activity influences this regulation. We propose that this intricate interplay between antibiotic, ribosome, and ABCF protein represents a novel mechanism that fine-tunes bacterial gene expression in response to antibiotic and thereby maintain their ability to survive in the natural environment.

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

P4

SOLUTION STRUCTURE OF cAMP-DEPENDENT PROTEIN KINASE RIIα SUBUNIT IN COMPLEX WITH MICROTUBULE ASSOCIATED PROTEIN 2c

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Intrinsically disordered proteins (IDPs) are a significant part of regulatory networks that control the cytoskeleton. Their interaction with kinases is key to understanding the mechanism of their function. Important IDPs in the brain include the MAP2/Tau family proteins. The ubiquitous cAMP-dependent protein kinase (PKA) also plays an important role in the function of brain neurons. Its localization is controled by A-kinase anchoring proteins (AKAPs), one of which is the microtubule associated protein 2c (MAP2c). The binding site for PKA on MAP2c was discovered a long time ago. However, structure of the complex had not been solved until now.

Here we present an atomic resolution structure of the interacting domains of the RIIα subunit of PKA in complex with MAP2c. The binding site was first confirmed using broadening of MAP2c NMR signals upon addition of PKA. Further relaxation and ensemble-based studies of free MAP2c indicated increased alpha-helical propensity for this region. ITC studies of the complex confirmed nanomolar binding affinity. For structure determination, the protein complex was prepared recombinantly with isotope labeling in both uniform and specific variants. NMR signals were assigned using tripple resonance and 3D NOESY spectra. Filtered 3D NOESY was used to probe intermolecular interactions in the complex. Residual dipolar couplings of NH pairs were measured using the Pf1 oriented phage. Distance restraints from 3D NOESY spectra were calculated using CYANA. The final structure was calculated using SCULPTOR-CNS.

We have solved the structure of MAP2c-PKA complex. The structure showed that intrinsically disordered protein MAP2c forms an α-helix upon binding to PKA similarly to other AKAPs. However, the orientation the helix differs from the other known AKAP structures. This finding significantly extends our undersanding of the principles that govern specific recognition of AKAPs by regulatory subunits of PKA.

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The work was supported by Czech Science Foundation, grant No. 20-12669S. Josef Dadok National NMR Centre, CF Biomolecular Interactions and Crystallography, and Proteomics Core Facility of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042) and European Regional Development Fund-Project „UP CIISB“ (No. CZ.02.1.01/0.0/0.0/18_046/0015974) are gratefully acknowledged for the access to NMR, ITC, and MS instruments. The authors thank Karel Kubíček for his help with CYANA structure calculations.

P5

INHIBITION OF HISTONE DEACETYLASE 6 BY 1,2, 4-OXIADIAZOLES: STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION

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Histone deacetylase (HDAC) inhibitors used in the clinic typically contain a hydroxamate zinc-binding group (ZBG). However, recent studies have shown that alternative ZBGs, including heterocyclic trifluoromethyl oxadiazoles (TMO), can confer greater isoenzyme selectivity and more favorable ADMET profiles. Here, we report the synthesis and biochemical and structural characterization of a series of HDAC inhibitors featuring 1,2,4-TMOs as ZBGs. Compared to 1,3,4-TMO inhibitors, incorporating the 1,2,4-TMO isomer into the inhibitory scaffold markedly alters selectivity, broadening activity across HDACs, including class IIa isoenzymes. Crystal structures of HDAC6-inhibitor complexes reveal that the 1,2,4-TMO ring of the parent oxadiazole undergoes hydro-

lysis within the enzyme's active site, yet the kinetics and catalytic mechanism differ from those observed for 1,3,4-TMO inhibitors. Overall, our findings provide compelling evidence that the choice of TMO warhead dictates not only HDAC isoform selectivity but also the transformation mechanism within target enzyme active sites.

The project was supported by the Czech Academy of Sciences (RVO: 86652036) and the Ministry of Education, Youth and Sports (MEYS - LUAUS23247).

P6

TARGETING THE mRNA CAPPING MACHINERY OF *TRICHOMANAS VAGINALIS* AS THERAPEUTIC APPROACH IN TREATMENT OF TRICHOMONIASIS

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Trichomonas vaginalis is a member of flagellated protist that causes trichomoniasis, the most prevalent nonviral sexually transmitted human infection. Globally, the estimated annual incidence is more than 270 million cases [1]. The extracellular parasite resides in the urogenital tract of both sexes and can cause vaginitis in women and urethritis in men. Acute infections are associated with increased risk of human HIV-1 infection, cervical carcinoma, infertility, prostatic adenocarcinomas and adverse pregnancy outcomes. Current treatment of trichomoniasis relies on administration of antiprotozoal drugs. However, resistance has been increasingly recognized and may occur in up to 10% of infections [2].

A promising path represents targeting a mRNA (messenger RNA) capping machinery of this parasitic protozoan. The 5' cap is an essential feature of eukaryotic mRNA that is required for a stability and efficient translation. mRNA capping entails three enzymatic reactions and last step is the cap methylation reaction, catalyzed by RNA (guanine-N7) methyltransferase (RNMT). TRIV-RNMT is a 39.8 kDa protein with 347 aa, localized in nucleus and shares 33% sequence identity to a bigger human cap-methylating enzyme (Hcm1: 476 aa), however, the human cap methyltransferase contains nonessential N-terminal extensions that are missing in TRIV-RNMT. *T. vaginalis* homolog protein contains S-adenosylmethionine (SAM) binding motif, structurally resembling human Hcm1.

Here we present a newly discovered crystal structure of this protozoan methyltransferase with a high-resolution (~1.9 Å) view of the catalytic site, occupied by a co-factor competitive compounds: Sinefungin and SAH (S-adenosylhomocysteine). These preliminary crystal structures accompanied with high-throughput screening of small inhibitory molecules (unpublished data) provide a valuable structural insight for consecutive rational drug design.

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This research was funded by project the National Institute of Virology and Bacteriology (Programme EXCELES. Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

P7

MONTE CARLO SIMULATIONS OF MINIPROTEIN FOLDING SAMPLED WITH AN AUTOENCODER

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Autoencoders are artificial neural networks that have been shown to be a high quality tool for non-linear dimensionality reduction and have many diverse applications. The architecture consists of two parts, an encoder, which transforms high dimensional input data onto a low-dimensional embedding, which is called latent space. Second part of the network, decoder, reconstructs these latent space values into the original high dimensional data.

In this work, we use structures of Tryptophan-cage miniprotein sampled from molecular dynamics (MD) simulation of high-temperature unfolding of the miniprotein, to train a simple autoencoder to be able to encode and decode miniprotein structures. Decoder part of the trained autoencoder can then be used to generate diverse protein structures based on input latent space values. The miniprotein structures generated this way include, but are

not limited only to the structures present in the training dataset.

We show that a well-trained decoder can generate structures with displacement of individual particles so small that, after a short potential energy minimization, the structures can be concatenated into a continuous trajectory based on Metropolis Criterion. Running such Monte-Carlo simulation of the miniprotein system provides a very computationally inexpensive way to visualize the structural behaviour of the studied miniprotein, including folding, unfolding and other events. At the same time, structures sampled along the Monte-Carlo trajectory form an ensemble that follows Boltzmann distribution. Although the distribution of structures sampled this way differs slightly from one sampled from sufficiently long classical MD simulation, it is also provided at a tiny fraction of the computational cost of the MD simulation.

P8

STRUCTURAL INSIGHTS INTO 14-3-3-MEDIATED REGULATION OF HUMAN ASK1

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Apoptosis signal regulating kinase 1 (ASK1), also known as MAP3K5, is a widely expressed member and a crucial stress sensor from the mitogen-activated protein kinase (MAPK) kinase kinase (MAP3K) family; directing cells toward apoptosis, differentiation, and senescence via the p38 and JNK signaling pathways [1]. Dysregulation of ASK1 has been associated with cancer, inflammatory, cardiovascular, and neurodegenerative diseases, among others. Hence, ASK1 activity has to be strictly regulated to respond stress stimuli appropriately. In unstressed condition, ASK1 is held in an inactive state through binding of negative regulators, such as thioredoxin and 14-3-3. Thioredoxin (Trx) is an oxidoreductase that participates in redox reactions and catalyzes dithio-disulfide exchange reactions whereas 14-3-3 is a highly conserved phosphoserine- and phosphothreonine-binding scaffold protein [2, 3]. Despite many years of intensive research, there is no atomic resolution structure of multi-domain ASK1 in complex with 14-3-3 or thioredoxin, which has hindered functional and mechanistic understanding of ASK1 regulation. Therefore, the main goal of this project is to unravel the structural and molecular basis of ASK1 regulation by the 14-3-3 protein using an integrated approach based on cryo-electron microscopy (cryo-EM), hydrogen- deuterium exchange coupled to mass spectrometry (HDX- MS), sedimentation velocity analytical ultracentrifugation (SV-AUC), and analytical size-exclusion chromatography (SEC).

The stability and stoichiometry of the ASK1:14-3-3 complex was investigated by SV-AUC. These experiments revealed that ASK1 and 14-3-3 form a complex with apparent dissociation constant (KD) of 90 ± 7 nM. The sedimentation coefficient distribution c(s) also suggested that the presence of 14-3-3 induced the tetramerization of ASK1, i.e. the formation of a complex with a stoichiometry of 4:4 (two ASK1 dimers forming a tetramer stabilized by two 14-3-3 dimers). The formation of this 4:4 complex was subsequently confirmed by SEC and cryo-EM. Cryo-EM reconstruction showed that each of the 14-3-3 dimers stabilizes the tetrameric arrangement of ASK1 by binding the C-terminal segments of ASK1 chains from opposite ASK1 dimers. This suggests that tetramerization of ASK1 causes steric hindrance of the catalytic centers of the kinase domains and presumably also interactions between the kinase domains and the MAP2K kinase substrate, thus explaining the inhibitory effect of 14-3-3 binding. Overall, our results provide the first insight into the structural basis of 14-3-3 protein-mediated regulation of ASK1 kinase.

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This study is supported by the Grant Agency of Charles University (JB: 151324) and the Czech Academy of Sciences (RVO: 67985823 of the Institute of Physiology). We acknowledge CMS-Biocev of CIISB, and Cryo-electron microscopy and tomography core facility CEITEC MU of CIISB, Instruct-CZ Centres, supported by MEYS CR (LM2023042).

P9

REPLICATION AND TRANSCRIPTION REGULATOR Rta OF EPSTEIN-BARR VIRUS - FUNCTIONAL AND STRUCTURAL IMPLICATIONS FOR NEW ANTIVIRAL STRATEGY

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The Epstein-Barr virus (EBV) is one of the most prevalent human viruses, infecting over 90% of the global population over the course of a lifetime. EBV is implicated in approximately 200 000 cancer cases annually and is associated with various premalignant lymphoproliferative disorders, including Hodgkin's lymphoma, gastric carcinoma, and nasopharyngeal carcinoma [1]. Beyond its oncogenic potential, EBV has been linked to infectious mononucleosis and multiple sclerosis [2,3].

The replication and transcription activator (Rta) is a key regulator of the EBV life cycle, as Rta mediates the transition from latency to the lytic phase. Rta functions as a transcriptional activator by binding to the Rta Response Element (RRE) on viral DNA, thereby initiating the expression of lytic genes, including the viral gene PAN [4]. Despite Rta functional significance, the structural properties of Rta remain uncharacterized, and no sequence homology has been identified between Rta and known DNA-binding or dimerization motifs [5].

In this study, we employed an interdisciplinary approach to elucidate the functional and structural properties of Rta. We characterized the biophysical attributes of Rta DNA-binding domain and examined Rta oligomerization. Additionally, we investigated the structural features of the DNA-binding domain and quantified Rta binding affinity for a DNA sequence containing the RRE motif. In parallel, we analyzed Rta function in a human cell line, focusing on Rta nuclear localization and sequestration from nucleoli.

Targeting Rta with small-molecule inhibitors represents a promising strategy for therapeutic intervention in EBV-associated diseases. Thus, a comprehensive understanding of Rta's structural organization and oligomeric state is crucial for the rational design of novel antiviral agents.

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The research was primarily supported by Czech Science Foundation (23-05241S). We acknowledge CEITEC Proteomics CF and CF Biomolecular Interactions and Crystallography of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042, e-INFRA CZ (ID:90254)) and European Regional Development Fund-Project „UP CIISB" (No. CZ.02.1.01/0.0/0.0/18_046/0015974). We acknowledge the CF CELLIM supported by MEYS CR (LM2023050 Czech-BioImaging) and Biological Data Management and Analysis CF funded by ELIXIR CZ research infrastructure (MEYS Grant No: LM2023055).

P10

DYNAMIC ALLOSTERIC REGULATION OF MYCOBACTERIAL INOSINE MONOPHOSPHATE DEHYDROGENASE

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Inosine-5'-monophosphate dehydrogenase (IMPDH) is a key enzyme in purine metabolism and a promising drug target against mycobacterial infections. Despite its central role at the crossroads of multiple branches of the purine metabolism, the regulation of mycobacterial IMPDH remains poorly understood. Here, we describe the molecular mechanism by which allosteric regulators dynamically modulate IMPDH activity in *Mycobacterium smegmatis*. Using single-particle cryo-EM, we obtained a comprehensive structural series of IMPDH in complex with substrates and regulatory molecules, including ATP, GTP, and the bacterial alarmone ppGpp. Our cryo-EM data, complemented by HDX-MS and SAXS experiments, reveal that these effectors exploit a common mechanism to alter the intrinsic dynamics of the octameric assemblies. By stabilizing distinct conformational states, they effectively trap IMPDH in a compressed, inactive conformation. This mechanism highlights a complex allosteric regulatory system that extends beyond simple feedback regulation, offering potential insights for the design of selective antimycobacterial drugs targeting IMPDH.

P11

UNRAVELLING THE MYSTERIES OF NOVEL TWO-DOMAIN LECTINS FROM OPPORTUNISTIC HUMAN PATHOGENS

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LecB (PA-IIL) is one of two characterised lectins (saccharide-binding proteins) from the bacterium *Pseudomonas aeruginosa*. Both proteins (LecA and LecB) play a significant role in bacterial infection and biofilm formation in immunocompromised patients (e.g. cystic fibrosis patients) [1]. Several LecB homologs were described in the past, for example, lectins produced by *Burkholderia cenocepacia* [2]. Nevertheless, there are still uncharacterised LecB-like proteins in the pathogenic bacteria, some of which contain an additional domain of unknown function. Their characterisation could provide insights into the mechanism of infection and lead to the development of novel approaches for disease treatment.

The aim of this project is the functional and structural characterisation of three potential two-domain lectins containing a LecB-like domain with an emphasis on their binding properties. The genes encoding these hypothetical carbohydrate-specific proteins were identified by bioinformatic analysis, cloned into expression vectors, and expressed in *Escherichia coli*. In addition, new gene constructs were prepared to characterise each domain separately. A variety of methods were used to investigate thermostability (nanoDSF), homogeneity (DLS, AUC) and binding properties (ITC, AUC) of the purified proteins. Several crystallisation screens were performed to obtain the crystals of the separate domains. The initial hits for X-ray crystallography are currently being optimised to obtain well diffracting crystals. For the whole proteins, electron microscopy methods are planned because of the expected high dynamics of the whole system.

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We acknowledge Core Facility Biomolecular Interactions and Crystallography of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042). This work was supported by the Czech Science Foundation (project 21-29622S).

P12

STRUCTURE OF DC11 FAB FRAGMENT SPECIFIC FOR THE PRE-AGGREGATION CONFORMATION OF IDP Tau

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A key yet unresolved question of the pathogenesis of Alzheimer's disease (AD) and other tauopathies is the cause and the mechanism of the transition from the unstructured monomeric tau protein to the insoluble filaments deposited in the brain tissue. In the physiological state, tau protein exists as a conformational ensemble of interconverting structures and on the scale of transition from monomeric through oligomeric and filamentous species we can observe conformations reacting with specific antibodies, mainly with DC11, which is able to specifically discriminate between tau proteins isolated from healthy brain and tau proteins isolated from the brain of AD patient. The antibody recognizes also the recombinant truncated tau proteins up to the shortest fragment tau321-391 [1].

It was found that conformational antibodies DC11 and MN423 have catalytic pro-aggregatory effects in tau aggregation assay, whereas the antibody DC8E8 has inhibitory effects on tau filament formation [2]. This may imply possible mechanism of induction of pathological tau conformation, in which the antibody prepared against pathological tau imprints the pathological conformation into the physiological tau proteins in solution and therefore speeds

up the tau aggregation. The information about conformational epitopes of these antibodies are therefore of high significance.

To further uncover the binding mode of the conformational antibody DC11, we have performed NMR epitope mapping using ^{13}C, ^{15}N labelled tau321-391 and tau297-391 (dGAE) and recombinantly prepared Fab fragment of DC11 antibody. The overlay of HSQC spectra showed the region of tau between residues 370-390 to be affected by the binding of DC11, i.e. its C-terminal region.

We have solved the X-ray structure of DC11 Fab fragment to a resolution of 1.33 Å and deposited it into the PDB database with PDB ID 9H8H. We have further measured the synchrotron SAXS data to characterize the conformational ensembles of tau321-391 and tau297-391 in both batch and SEC-SAXS modes. We have also attempted to characterize the complexes between tau proteins and DC11 Fab fragment.

The results highlight the importance of the R' region of tau, that was recently shown to be important also for tau interaction with microtubules [3]. This sequence forms the interface of rigid filament core and flanking fuzzy C terminal segment in solved tauopathy filaments.

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This research was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00623. This work was supported by research grants APVV 21-0479, VEGA 2/0125/23 and 2/0141/23. This research was further funded by the European Union's Horizon Europe program under the grant agreement No. 101087124 and MSCA-RISE grant No. 873127.

P13

GENOME ANCHORING, RETENTION, AND RELEASE BY NECK PROTEINS OF *STAPHYLOCOCCUS* PHAGE 812

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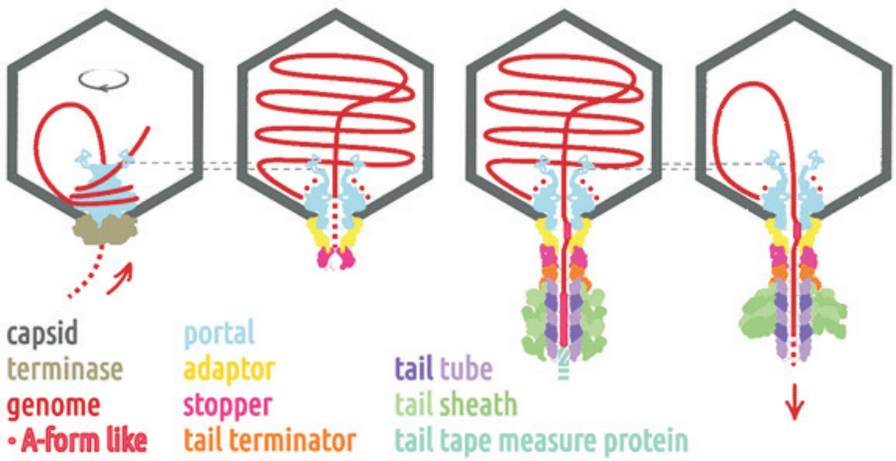
The virion of *Staphylococcus* phage 812 is formed by an icosahedral capsid and a contractile tail joined together by neck proteins. Despite the role of the neck proteins in virion assembly, DNA packaging, and regulation of genome release, their functions are not well characterized. Here we show that the neck of phage 812 consists of portal, adaptor, stopper, and tail terminator proteins decorated on the outside by two types of cement proteins. A dodecameric DNA-binding site on the portal complex anchors the genomic terminus inside the capsid, which may prevent an accidental escape of the DNA during the initial stages of genome packaging. The adaptor complex induces a local B-to-A form transition of the DNA in the neck channel that may serve to pause genome translocation. The gating loops of the stopper proteins prevent genome loss from fully packaged proheads by blocking the neck channel prior to the tail attachment. The binding of the tail terminator complex to the stopper complex induces opening of the gating loops and advancement of DNA into the tail. The structure of neck proteins is unchanged by tail sheath contraction. The expulsion of the tail tape measure protein rather than tail sheath rearrangement thus triggers genome release. Our results explain how the active interplay between neck proteins and the genome directs DNA packaging, prevents



premature genome release, and enables its ejection into the host cell.

The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

P14

BIOPHYSICAL CHARACTERIZATION OF BIOMOLECULES AT THE CENTRE OF MOLECULAR STRUCTURE

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Biophysical research facility of the Centre of molecular structure is a shared resource for the characterization of biomolecules that provides an access to instruments, technologies, expert consultation and training to researchers.

For the determination of size, molecular mass, structure and stability of biomolecules, study of conformational changes and thermodynamics of temperature transitions are currently available: mass photometry (Two MP mass photometer) circular dichroism spectroscopy (Chirascan Plus CD spectrometer), spectrophotometry (Specord 50 Plus UV/Vis spectrophotometer), Fourier-transform infrared spectrometry (Vertex 70v spectrometer), fluorescence spectrometry (photoluminescence spectrometer FLS1000), differential scanning fluorescence (Prometheus NT.48), multiangle dynamic light scattering (Zetasizer Ultra), microplate reader (Tecan), differential scanning calorimetry (Microcal VP-DSC). Isothermal titration calorimetry (Microcal iTC200 and PEAQ-ITC), microscale thermophoresis (Monolith NT.115 and NT.LabelFree), surface plasmon resonance (ProteOn XPR36) and bio-layer Interferometry (OCTET R8) techniques are available for the characterization of biomolecular interactions.

Facility is a member of Instruct-ERIC, Czech Infrastructure for Integrative Structural Biology (CIISB) and Molecular-Scale Biophysics Research Infrastructure (MOSBRI).

All relevant information is on the web pages: https://www.ibt.cas.cz/cs/servisni-pracoviste/centrum-molekularni-struktury/, https://www.ciisb.org/open-access/core-facilities.

The Centre of Molecular Structure is supported by: MEYS CR (LM2023042); project UP CIISB (CZ.02.1.01/0.0/0.0/18_046/0015974), CIISB4HEALTH (CZ.02.1.01/0.0/0.0/16_013/0001776); ELIBIO (CZ.02.1.01/0.0/0.0/15_003/0000447), and MOSBRI (No. 101004806).

P15

IDENTIFICATION OF A NOVEL ERYTHROMYCIN RESISTANCE MECHANISM MEDIATED BY MrmA METHYLTRANSFERASE IN *C. DIFFICILE*

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Clostrioides difficile is one of the main causes of hospital-acquired diarrhea [1]. However, the accumulation of antimicrobial resistance in epidemic *C. difficile* lineages such as RT176 poses a significant risk for the spread of resistance determinants to other bacterial species targeted by these antibiotics. A comparative genomic analysis of one erythromycin-susceptible and six erythromycin-resistant *C. difficile* strains identified the novel resistance determinant carried by transposons Tn6110 and Tn7806 for which we propose the name mrmA (macrolide resistance methyltransferase A). We demonstrated that heterologous expression of the mrmA gene in *E. coli* confers resistance to erythromycin and to a lesser extent to streptogramin B, but not to other ribosome-targeting antibiotics.

MrmA encodes a putative SAM-radical 23S rRNA methyltransferase, similar to RlmN and Cfr [2]. RlmN is a housekeeping enzyme involved in translation fidelity and methylates nucleotide A2503 at carbon C23. Cfr-mediated methylation at the same A2503, but at carbon C8, confers resistance to antibiotics targeting the peptidyltransferase center (PTC) [3]: phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A, with no effect on erythromycin activity. In contrast, resistance to erythromycin is generally conferred by a different Erm-family of 23S rRNA methyltransferases [3]. These Erm enzymes dimethylate nucleotide A2058 in the exit tunnel, resulting in resistance to macrolides, lincosamides and streptogramins B antibiotics.

We hypothesize that MrmA methylates a different adenine residue on the 23S rRNA compared to Cfr and RlmN, which selectively affects erythromycin binding without affecting oxazolidinones or lincosamides [3]. To elucidate the exact site of modification, we will use *in vitro* biochemical and structural biology approaches, which we will present in detail.

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The work was supported by the project National Institute of Virology and Bacteriology (ProgrammeEXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - NextGenerationEU.

P16

STRUCTURAL STUDIES OF BIOLOGICALLY RELEVANT PROTEIN VARIANT OF CANCER-RELATED CARBONIC ANHYDRASE IX

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During tumor development cancer cells express various proteins to proliferate, overcome the unfavorable conditions within the tumor environment and further progress through the body. Each of these processes is enhanced by overexpression of enzyme carbonic anhydrase IX (CA IX) on cellular surface, making it an attractive target for the development of anticancer therapy. However, the discovery of specific drug compound has been hindered by existence of other fourteen CA isoforms within the human body and the fact that CA IX high-yield production and reproducible crystallization has been challenging. For that, number of protein variants have been established to ease the process of structure-based drug design, though some of them have lost the CA IX's property of dimer formation. Here, we describe a prospective workflow of heterologous production of recombinant CA IX extracellular region from *Escherichia coli*. Importantly, the biophysical characterization and crystal structure revealed that the biologically relevant dimeric arrangement is preserved and disclosed residues crucial for maintaining the dimeric interface. This CA IX variant thus represents valuable tools to reproducibly produce and crystallize biologically relevant dimeric form of CA IX.

P17

INFLUENCE OF THE REPARAMETRISATION OF THE PROTEIN-WATER INTERACTIONS ON THE CONFORMATION ENSEMBLE OF Tau(210-240)

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Tau is a well-known intrinsically disordered protein (IDP), regulating the assembly and maintenance of the microtubules. [1] However, under pathological conditions, tau undergoes various hyperphosphorylations. Those modified tau species detach from the microtubules, fibrillise and assemble into neurofibrillary tangles, the main pathologicalhallmarks of Alzheimer's disease (AD) in human brains. [2]

Understanding the mechanism of tau fibrillation is the key to understand the neurodegenerative process of AD. Molecular dynamics (MD) simulations can provide insights on the time-evolution of tau fibril formation. Coarse-grained (CG) simulations allow to extend the lengths and time scale of the simulations by orders of magnitudes compared to the all-atom resolution. [3] Accurate CG force fields are necessary for the description of Tau proteins in MD simulations. However, CG force fields tend to underestimate the global flexibility of IDPs and result in conformations, that are too collapsed. [3]

The accuracy of the CG force fields SIRAH 2 [4] and Martini 3 [5] for the description of the monomeric ensemble of Tau(210-240) is investigated. The accuracy of the CG force fields is evaluated by comparison with atomistic simulations. Furthermore, experimental results, such as

NMR data and the radius of gyration is used to validate the accuracy of the CG force fields. The unmodified SIRAH 2 and Martini 3 force fields are resulting in too collapsed structures. The collapsing nature of the CG force fields is shown to be overcome by strengthening the water-protein interactions. The reparametrised CG force fields give a fair description of the conformational ensemble of Tau(210-240) and can accurately reproduce experimental data.

The computational resources of IT4Innovations were granted by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90254). This project was supported by the Brno Ph.D. Talent Scholarship, funded by the Brno City Municipality and by the European Union's Horizon Europe 2020 program under the grant agreement No. 101087124 - ADDIT-CE.

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P18

INVESTIGATING NUCLEOSOME SPACING AS AN EPIGENETIC REGULATORY MECHANISM

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Nucleosomes are complexes of histone proteins and DNA formed by eukaryotic cells whose function is to prevent non-specific aggregation of DNA by allowing for controlled condensation in the form of chromatin as well as regulation of transcription and other processes by either physically blocking parts of DNA or, depending on its posttranslational modifications (PTMs), recruiting the transcription machinery [1]. In chromatin, nucleosomes typically form arrays in which the distance between neighbouring nucleosomes (the average length of histone-free DNA linker) is relatively constant. While the presence of regular arrays is a feature of chromatin conserved though all eukaryotic organisms, the average length of DNA linkers differ between organisms, and even between different cell types of one organism [2]. Although several factors are known to affect the positioning of nucleosomes, the biological function of their differential spacing remains poorly understood [3].

Previous research has shown that linker length and even spacing in chromatin influence the formation of higher-order chromatin structures. These structural variations can affect the accessibility of transcription machinery and even contribute to the formation of phase-separated condensates [4]. Additionally, several enzymes have been identified with a preference for binding di-nucleosomes over mononucleosomes, where the length of the DNA linker affects their activity [2]. Based on these findings, we hypothesize that linker DNA serves as an additional layer of epigenetic regulation, with its length acting as a discriminating factor for the binding of chromatin-associated factors.

We have prepared a DNA construct library with variable linker lengths covering all the typical10n+5 as well as less common 10n options and ranging from 10 to 35 bp. Upon reconstitution of the nucleosomes and immobilization on streptavidin beads, these constructs will be used to pull down protein binders specific for each linker length from nuclear lysates. The bound proteins will be identified by mass spectrometry based proteomics and selected binder will be selected for further structural characterization.

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P19

STRUCTURAL, SOLVENT, AND TEMPERATURE EFFECTS ON PROTEIN JUNCTION CONDUCTANCE

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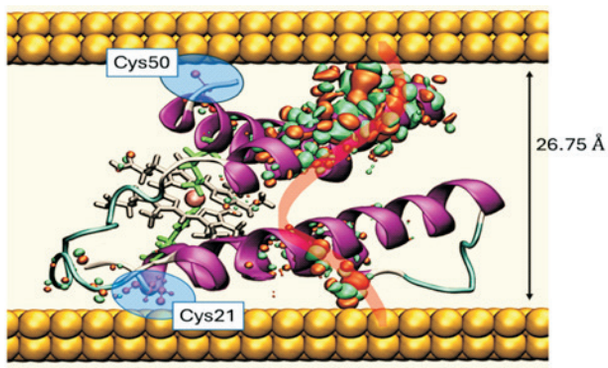
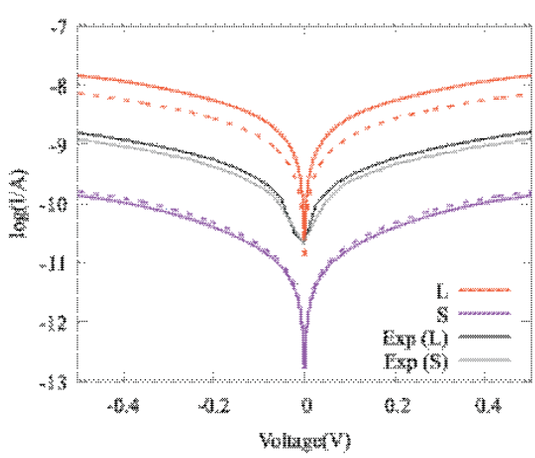
Electronic conductance of redox proteins in their native environment is typically facilitated by the organometallic cofactors, like hemes in cytochromes, which can be reversibly reduced / oxidized. The electrons thus flow through such proteins by sequential hopping that can be well described by the Marcus theory. However, when these proteins are immobilized on metal surfaces, and their conductance is probed by a scanning tunneling microscope (STM) or its electrochemical variant (EC-STM), magnitudes, shapes, and temperature dependencies of the measured current-voltage curves suggest coherent tunneling as the undergoing transport mechanism rather than hopping. [1-3] To elucidate these data and investigate the factors affecting charge transport in biomolecular junctions, we developed a computational procedure based on multiscale modeling involving classical molecular dynamics (MD), electronic-state calculations within density functional theory (DFT), and electronic coupling calculations. [4-6] Here, we demonstrate its feasibility in the study of single-heme cytochrome b_{562}, [7] for which the EC-STM data were previously reported in the literature.

Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructure.



a)



b)

Figure 1: Cytochrome b_{562} junction: (a) representative structure with highlighted chemisorbed cysteines and highlighted conduction channel; (b) computed tunneling current curves for different structures with indicated solvent-screening effects.

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P20

ASYMMETRIC PARTICLES OF TBEV PROVIDE INSIGHT INTO MECHANISMS OF FLAVIVIRUS ASSEMBLY AND MATURATION

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Tick-borne encephalitis virus (TBEV), an enveloped virus belonging to the *Flaviviridae* family, causes severe central nervous system disease in humans. The virus has a smooth surface covered by envelope proteins (E-proteins), which, along with membrane proteins (M-proteins), are anchored in the viral lipid bilayer. During its life cycle, the immature, non-infectious virus undergoes a maturation process characterized by the proteolytic cleavage of prM and significant rearrangement of the envelope proteins on its surface.

We isolated immature TBEV particles from infected tissue culture cells and visualized their structure using cryo-electron microscopy. We solved the high-resolution structure of the E-protein-prM-protein complex, which forms the "spiky" surface of immature particles. Through combination of cryo-electron tomography and single-particle analysis, we demonstrated that TBEV immature particles are asymmetric. Assembly defects often disrupt the symmetric, icosahedral structure of the E-protein-prM-protein spikes on the particle surface. However, these irregularities do not impede the subsequent maturation process, resulting in mature particles with vacant patches in the "herringbone" pattern of the mature viral surface.

The findings shed additional light on the viral assembly of TBEV and its maturation process, which may be the subject of future antiviral medication development.

This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

P21

STRUCTURAL CHARACTERIZATION OF AIRE AND ITS INTERACTIONS

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The Autoimmune Regulator (AIRE) is essential for maintaining immune tolerance by promoting the expression of tissue-specific antigens (TSAs) in thymic medullary epithelial cells [1]. Mutations in AIRE are linked to APECED (Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy), a severe multi-organ autoimmune disorder [2].

AIRE consists of multiple domains interspersed with intrinsically disordered regions (IDRs), which together facilitate its interactions with chromatin and transcriptional regulators. While significant progress has been made in understanding AIRE's physiological role, the molecular mechanisms underlying its transcriptional regulation function remain unclear.

To investigate AIRE's interactions, we prepared a panel of AIREs constucts. These constructs will be used in pull-down assays followed by mass spectrometry to identify interacting partners. We will further characterize these interactions structurally and biophysically to gain deeper insights into the molecular basis of AIRE function.

A more detailed understanding of the interactions between AIRE and its partners will provide valuable insights into the molecular mechanisms of transcriptional regulation in eukaryotes and immune tolerance.

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P22

SEARCH FOR PATHOLOGY-INDUCING *IN VITRO* Tau FILAMENTS

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Tau protein, predominantly found in the central nervous system, plays diverse roles in neurons, including microtubule regulation, signal transduction, and fast axonal transport. However, misregulated tau forms pathological filaments, which are a hallmark of neurodegenerative diseases like Alzheimer's disease. Aggregation and accumulation of misregulated tau is toxic to the neurons. Despite intense efforts of scientific community, the underlying mechanisms of tau pathology are still not well understood [1].

Advances in amyloid cryo-EM helical reconstruction have revealed distinct structural differences in tau filaments among various diseases [2]. However, the mechanism of tau aggregation remains unknown, and the reason behind conformational diversity is not yet clear.

Currently, disease-like tau filaments can be prepared in vitro using dGAE (297-391) tau fragment and phosphomimicking tau constructs [3-5]. They have higher aggregation propensity and readily form filaments without aggregation inducers. Interestingly, their conformation highly depends on buffer conditions.

Our goal is to understand the factors influencing and driving tau aggregation and to prepare in vitro recombinant tau filaments. We are preparing tau filaments using tau isoform 2N4R and dGAE tau fragment in different buffer conditions (salt content, pH). Filaments are analysed using negative stain EM, and cryo-EM. Here we present our preliminary results of the ongoing study and discuss the aggregation protocol, morphological differences, and biological relevance of the prepared filaments.

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P23

UNDERSTANDING THE PROTEIN CRYSTALLIZATION

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Current Practice of Protein Crystallization

Already a half century, thousands of laboratories around the world are routinely using the protein crystallization as the most accurate method for experimental determination of the structure of proteins and their complexes. The diffraction methods on the small-molecular organic crystals allow the structure determination in subatomic resolution and often offering the observation of atom orbitals. But the accuracy in protein crystallography is pretty much worse mostly because a very low quality of protein crystals. In addition, a number of mysteries without any reasonable explanation surrounds the protein crystallization.

Practical crystallization is de facto the method of trials and errors. Crystallization robots set thousands of crystallization conditions that have been proven useful in the past for some other proteins and their complexes. Theoretical works describing protein crystallization using only classical thermodynamics slip usually into clarification of the precipitation rate. They do not explain well the function of so-called crystallization additives that were experimentally proved as necessary for a success of crystallization. Namely, the classical theory also do not solve clearly the fundamental problem of crystallographers, i.e. why the precipitation results in a crystalline phase or in a non-diffracting amorphous precipitate. Our approach adds some new aspects including the kinetics of molecules in the su-

persaturated crystallization solution important for the error-free stacking of protein molecules into the growing crystal.

Dynamic Theory of Protein Crystallization

Large surface of protein molecule offers many possible adhesion modes between molecules. The adhesion modes mutually incompatible in a single crystal form lead to stacking faults disabling a growth of the regular crystal. Any successful method of protein crystallization can be explained by a reduction of the incompatible adhesion modes. Understanding how it works requires an analysis of physical processes acting during temporal clustering of molecules in the molecularly overcrowded crystallization solution close to saturation. An important role plays the orientation of the protein-additive clusters during their motion in the saturated crystallization solution before their deposition on the crystal surface. The additives can also block the deposition of protein molecules in the incompatible adhesion modes. The molecules of additives are usually pressed out of the growing crystal. However sometimes, they are preserved in their interaction with protein in the crystals serving thus as an excellent experimental proof of our theory [1-4].

As far as the initiation of crystallization by the hetero-surfaces immersed in solution, it is explained by formation of very stable error-free crystal-seeds on the hetero-surface. The stable seeds can survive and can grow even under the conditions where the spontaneously formed irregular-crystal-seeds in the bulk solution dissolve.

The differences in comparison with the classical theory include:

- formation of temporal (meta-stable) clusters of protein molecules with other molecules in crystallization solution (additives).
- changes of adhesive patches on the protein surface temporarily induced by the clustering.
- recognizing the mechanical forces accompanying the molecular motion of protein clusters in the solution close to saturation.
- recognizing the preferred orientation of supramolecular clusters during their movement in the molecularly overcrowded crystallization solution close to saturation.

Our dynamic theory of protein crystallization explains all common problems and mysterious behavior of uncountable number of crystallization experiments. It analyses formation of temporary supramolecular adducts and their motion in the saturated crystallization solution. This new approach uses the physical forces that are not commonly used in chemical and biochemical sciences. However, the formation of the supramolecular adducts is experimentally well confirmed by thousands of experiments in the PDB, as it is shown for example in [1-4]. The validity of models describing their kinetics is seen in that they fully explain thousands of experimental crystallization experiments.

Conclusion

Rational approach to crystallization based on the dynamic theory of protein crystallization and on the theory of adhesion modes can be used:

- for design of optimal crystallization screens tailored to a specific protein,
- to increase the quality of crystals leading thus to a better resolution of structure determination,
- to increase the efficiency of crystallization screens and lower consumption of protein sample,
- to explain the function of the protein crystal catalysers.

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P24

WHEN SIGNALING GOES ASTRAY: UNDERSTANDING MAP2K1 MUTATIONS IN NEURODEVELOPMENTAL DISORDERS

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The RAS/MAPK signalling pathway is one of the most extensively studied pathways, mainly due to its fundamental role in the regulation of cell cycle, proliferation, or senescence. Mutations in RAS/MAPK are primary drivers of cancer [1]. They also contribute to developmental syndromes known as RASopathies. These syndromes are associated with body malformations of different severity and with impaired cognitive function. Individuals with these syndromes often experience intellectual disability (ID) and autism spectrum disorder (ASD) [2, 3]. In this study, we investigate *de novo* recurrent single-point missense mutations in *MAP2K1* gene (encoding MEK1 protein), found in individuals with ID and ASD. They are currently classified as variants of unknown significance (VUS) [4]. It is crucial to investigate whether and how they impact the protein function, as some variants in RASopathies are known to increase or decreas the kinase activity.

The variants were selected from large sequencing studies [4] using an initial dataset of all missense variants found in ID/ASD individuals. We considered the number of affected individuals with each variant and the presence of secondary mutations in other genes. Further, the variants were cross-validated for their association with ID or ASD with ClinVar Miner (https://clinvarminer.genetics.utah.edu/), SysNDD (https://sysndd.dbmr.unibe.ch/) and SFARI database (https://gene.sfari.org/).

We cloned the wild-type MEK1 (MEK1wt), and the corresponding VUS-containing variants. We express and purify the proteins for assessment of kinase activity and for investigating the effect of VUS on 3D structure with X-ray crystallography. This study aims to elucidate the pathogenicity of the novel mutations and the molecular mechanisms by which they lead to ID/ASD. This will help to improve diagnostics and find tailored treatments targets.

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This work is supported by by a grant from the Czech Science Foundation (grant no. 23-07810S) and an EMBO Installation grant (grant no. IG-5310-2023) to M. Fenckova.

P25

NEW KIDS ON THE BLOCK: UPDATE ON EQUIPMENT INSTALLED AT BIC CORE FACILITY, CEITEC, BRNO

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The Core Facility Biomolecular Interactions and Crystallography at CEITEC MU in Brno serves as a central hub for biophysical analysis of proteins and nucleic acids, being visited by almost a hundred of scientists and students every year. Since the methodology in the field of biomacromolecular studies develops rapidly, it is necessary to perform regular upgrades of the instrumentation as well as acquiring new machines. Over the last year, several new pieces of equipment have been installed and are now available to the users.

Mass photometer TwoMP (Refeyn) is designed to precisely determine molecular mass of proteins, nucleic acids and other macromolecules in a broad range of sizes. The machine is equipped with a recently developed fluidics

system, allowing to analyze both high- and low-stability complexes across various concentrations down to nM range. Modular spectrofluorometer Fluorolog QM (Horriba) enables various fluorescence measurements including fluorescence anisotropy and time-resolved anisotropy with time-correlated single photon counting. CD spectrometer Chirascan V100 (Applied Photophysics) is essential for investigating the 2D structure and stability of proteins, nucleic acids, and chiral drugs. It replaced the previous system, providing higher signal to noise ratio and sensitivity. The analytical SEC instrument OmniSEC (Malvern Panalytical) has been equipped with a fraction collector, allowing for subsequent analysis of the samples using highly sensitive techniques, e.g. mass spectrometry or electron microscopy.

Measurements at CF Biomolecular Interactions and Crystallography of CIISB, Instruct-CZ Centre are supported by MEYS CR (LM2023042) and European Regional Development Fund-Project „UP CIISB" (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

P26

STRUCTURAL VARIABILITY OF PEPTIDE DEFORMYLASE

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The first enzyme encountered by bacterial proteins after synthesis is peptide deformylase (PDF). PDF binds to the ribosomal surface and removes the formyl group from the N terminus of the nascent protein as it emerges from the exit tunnel. Based on structural and sequence similarity, PDFs are divided into Type I, II, and III, with Type I being further divided into subgroups IA and IB. Type I PDFs feature a C-terminal α-helix that serves as the connection point between the PDF's catalytic domain and the ribosome's surface. Conversely, Type II PDFs exhibit an intrinsically disordered C-terminal region and the mechanism by which Type II PDFs bind to the ribosome is unknown. Due to sequence divergence in otherwise conserved motifs of Type I and II, Type III PDFs are presumed to be inactive. Until recently, PDFs were thought to exist only in bacteria. However, eukaryotic PDF analogues have since been identified in plant and mammalian cells. Nevertheless, the role of PDF in mammalian cells remains unclear.

In our study, we investigate the folding behaviour of the C-terminal region by conducting all-atom molecular dynamics simulations of PDFs derived from various organisms. We selected five representatives of prokaryotic PDFs and two eukaryotic PDFs: a plastidial representative and an enzyme that was synthesized to resemble the human mitochondrial PDF. Our results suggest a high degree of similarity between bacterial and eukaryotic PDFs, particularly in classification, C-terminal flexibility, and structural resemblance.

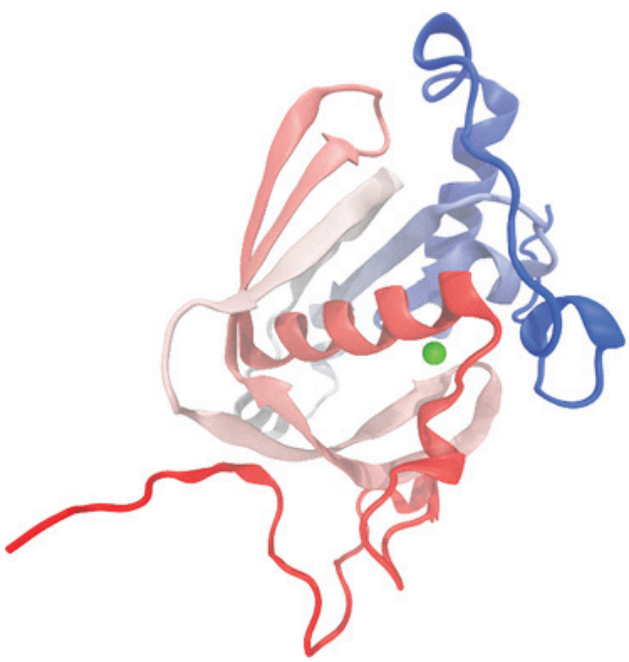

Figure 1. Human mitochondrial PDF analogue, 4JE6.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90254), and by the Czech Science Foundation (project 23-05764S).

P27

LEAKING WATER INTO A TEM: METHODS TO OVERCOME PREFERRED ORIENTATION IN CRYO-EM

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Preferred orientation is a frequently encountered problem in current single particle cryo-electron microscopy (cryo-EM) [1]. Proteins often adsorb to the air-water interface in a limited number of orientations, which subsequently results in sparsely populated or completely missing views during imaging in the electron microscope. Consequently, obtaining high resolution reconstructions may be difficult or impossible altogether [2].

Previously, we observed a reduction of preferred orientation after rapid laser flash melting and revitrification of samples containing highly symmetric and large particles [3-5]. Here, we expand this observation on varying systems, including T20S proteasome, 50S ribosomal subunit and HIV-1 Envelope protein.

Cryo-EM samples are locally flash-melted using a laser pulse, which exerts small forces on the protein particles, detaching them from the air-water interface and scrambling their orientations. When the laser is switched off, the sample rapidly revitrifies, trapping the particles in their newly adopted orientations [6, 7].

Our experiments show that the changes of angular distributions result from two competing processes. First, particles must be detached from the interface to even allow for their free rotation and hence adopt the new orientations. Simultaneously though, particles may diffuse back to the interface where they can once again settle into their preferred orientation.

An experiment involving *in-situ* deposition of amorphous ice onto the cryo-EM specimen prior to laser flash melting allows us to separate these processes [8]. We deposit 20 nm of amorphous ice by dosing water vapor into the specimen region inside a modified transmission electron microscope. When the sample is subsequently flash melted, particles find themselves not attached to the interface, but surrounded by liquid from all sides. Therefore, the resulting angular distribution reflects only the result of their free diffusion.

These experiments provide a set of tools to change and reduce preferred orientation of a wide range of systems, allowing for more reliable reconstructions as well as, in some cases, improvement of resolution. Importantly, the method does not require any extensive changes to the sample preparation and can be integrated into the existing workflows.

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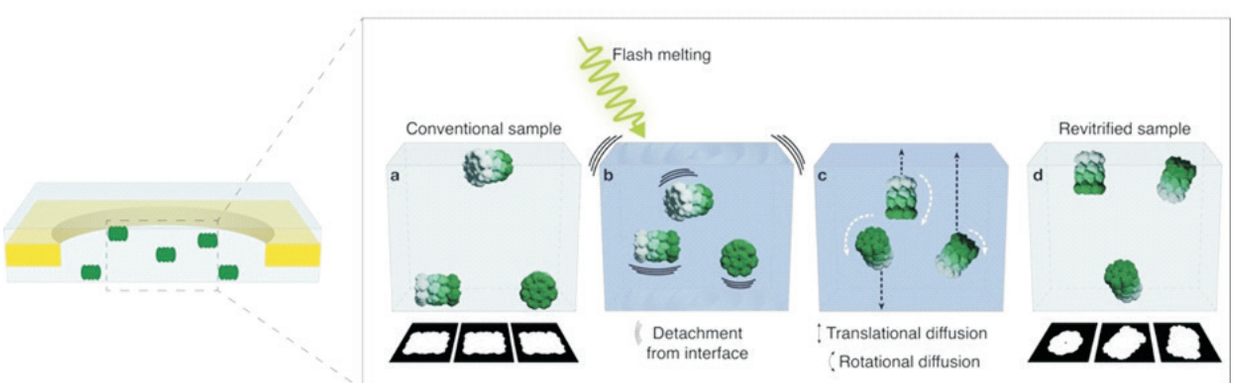

Figure 1. Mechanism of protein reorientation after laser flash melting and revitrification. T20S proteasome (PDB ID: 6BDF) is shown as an example. **(a)** In a conventional sample, particles adsorb to air-water interface, leading to limited viewing angles during imaging. **(b)** Laser flash melting detaches the particles from the interface and scrambles their orientation. **(c)** While the sample is kept liquid, particles are free to rotate and diffuse, adopting broader range of orientations. **(d)** Particles diffuse back to the interface over the time scale of our experiment, where they adopt new orientations. After revitrification, the particles are trapped in these orientations, resulting in more diverse viewing angles during imaging.

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P28

ENHANCING PETASE EFFICIENCY: A UNIFIED APPROACH TO PRODUCTION AND ACTIVITY ASSESSMENT

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The enzymatic degradation of polyethylene terephthalate (PET) has gained increasing attention as a promising strategy for plastic waste management. PETases, first discovered in *Ideonella sakaiensis*, have since been optimized through both natural evolution and protein engineering to enhance their efficiency and stability. However, comparing PETase variants across studies remains challenging due to inconsistencies in enzyme production, reaction conditions, and analytical methods.

To address this issue, we have developed a standardized protocol for PETase production, including expression, purification, and quality control steps. Building upon this standardized production protocol, we have systematically benchmarked the activity of five PETase variants with different thermal stabilities under a range of experimental conditions. Our study evaluates key factors such as enzyme concentration, reaction conditions, and analytical methodologies to establish a robust framework for assessing PET-degrading enzymes. Additionally, we employed deep learning-based tools trained on hyperthermophilic proteins to design a novel thermostable PETase variant. This AI-generated enzyme is currently undergoing experimental validation to determine its catalytic efficiency and stability compared to existing PETases.

By combining standardized production methods, systematic benchmarking, and AI-driven enzyme design, our work provides a comprehensive strategy to advance plastic degradation technologies. This approach lays the foundation for accelerating research in enzymatic PET degradation and its applications in plastic waste management.

P29

TARGETING 3Cpro: A NOVEL STRATEGY FOR EFFECTIVE ENTEROVIRUS D68 INHIBITION

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Enterovirus D68 (EV-D68), a member of the *Picornaviridae* family, has emerged as a significant pathogen associated with severe respiratory illnesses and neurological complications, particularly in children. Notably, EV-D68 has been linked to acute flaccid myelitis (AFM), a condition affecting the gray matter of the spinal cord and resulting in polio-like neurological symptoms such as muscle weakness and paralysis. A critical component of the EV-D68 life cycle is the 3C protease (3Cpro), which processes the viral polyprotein into functional units essential for viral replication and maturation [1, 2], making it an attractive target for antiviral drug development. Both covalent and non-covalent inhibitors of 3Cpro have shown promise in preclinical studies, though challenges related to resistance, specificity, and drug delivery remain [3, 4].

In this study, we present the high-resolution crystal structure of the EV-D68 3Cpro in complex with a novel inhibitor, RHCDS1a, resolved at 1.81 Å resolution. The gene encoding 3Cpro was cloned, overexpressed in *E. coli*, and purified using affinity and size-exclusion chromatography to obtain a stable and highly pure protein sample. Biochemical characterization revealed the inhibitory efficacy of RHCDS1a, with an IC_{50} of 550 ± 43 nM as determined by fluorometric protease inhibition assays. Surface plasmon resonance (SPR) analysis confirmed the strong binding af-

finity between RHCDS1a and 3Cpro, with a dissociation constant (K_D) of 766 ± 2 nM.

Structural analysis of the 3Cpro-RHCDS1a complex revealed that the inhibitor binds to the active site of 3Cpro, interacting with key residues within the catalytic triad (Cys147, His40, and Glu71) and the substrate-binding pocket. These interactions effectively disrupt protease activity. The inhibitor's imidazole ring exhibits two alternative conformations, suggesting opportunities for further optimization to enhance its potency and selectivity.

Our findings provide a detailed structural basis for the development of RHCDS1a as a therapeutic candidate against EV-D68. The insights gained from this study offer valuable guidance for the rational design of next-generation antivirals targeting 3Cpro, highlighting the importance of structure-based drug design in combating emerging viral pathogens.

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This research was funded by the European Union under the Horizon Europe, PANVIPREP/ Developing broad-spectrum antiviral drugs for pandemic preparedness (Grant Agreement No. 101137229). We gratefully acknowledge the support provided by the EU in enabling this study.

P30

STRUCTURAL CHARACTERIZATION OF THE INTERACTION BETWEEN BRCA1-BARD1 AND RNA POLYMERASE II

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Transcription competes with other DNA-dependent processes, such as DNA repair and replication, for access to its substrate, DNA. However, the principles governing the interplay between these processes remain poorly understood. Evidence suggests that the BRCA1-BARD1 complex, a key player in the DNA damage response, may act as a mediator of this crosstalk. The BRCA1-BARD1 complex is involved in multiple aspects of double-strand break (DSB) repair, protection of stalled replication forks, and the prevention of transcription-replication conflicts. Notably, BRCA1-BARD1 has been reported to interact with RNA polymerase II (RNAPII), yet the functional significance of this interaction remains unclear.

In our study, we investigated the molecular mechanism of the interaction between RNA polymerase II (RNAPII) and the BRCA1-BARD1 complex, as well as its functional consequence. Our data suggest BRCA1-BARD1 directly interacts with RNAPII through the binding of its BRCT domains to the phosphorylated C-terminal domain (CTD) of RNAPII. Moreover, we show that this interaction is critical for the organization of RNAPII into condensates with liquid-like properties. Analysis of disease-associated variants within the BRCT repeats further supports the biological relevance of this condensation process.

Collectively, these findings suggest that BRCA1-BARD1 may function as a molecular bridge between transcription and DNA repair pathways, facilitating crosstalk between these processes at sites of DNA damage and transcription-replication conflicts. The formation of liquid-like condensates may represent the underlying mechanism through which BRCA1-BARD1 mediates this crucial role.

P31

USING MASS PHOTOMETRY FOR SORTING AND SELECTION OF NATIVELY PURIFIED PROTEIN COMPLEXES

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Mass photometry (MP) is a relatively new biophysical technique based on the principles of the interference reflection microscopy. It measures the interferometric scattering signal of individual particles (macromolecules). It is perfectly suited for the direct molecular weight measurement of biological macromolecules in the solution without any labeling or prior immobilization in a wide interval of masses (from ~ 50 kDa to 5 MDa). The measurement itself is relatively inexpensive, fast and user-friendly. The main applications of MP are 1. Testing of samples for purity and monodispersity. 2. Analysis of oligomerization and formation of complexes. 3. Quality and stability control (e.g. aggregation in the sample over time) [1]. MP can also be used for the analysis of biomolecular interactions including the estimation of K_D, but these applications require special conditions and more considerations [2].

In our studies of the bacterial transcription machinery, we often isolate complexes of RNA polymerase (RNAP) directly from the native source (e.g. *B. subtilis*). Naturally, such samples contain complexes of RNAP with various transcription factors and also nucleic acids. Based on the growth conditions and processing procedures, we can to some extent control the enrichment of the sample by the complexes of our interest, nevertheless, these solutions contain several different "species" of RNAP even after multi-step purification, including size exclusion chromatography. We have found that the analysis of the post-purification fractions using MP helps us to select fractions with the highest possible uniformity and thus prepare the most suitable samples for follow-up structure-function and biophysical analyses.

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P32

STRUCTURAL BASES OF "COPY-OUT-PASTE-IN" TRANSPOSITION

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The copy-out-paste-in transposition mechanism is a major replicative pathway used by many prokaryotic transposable elements. In contrast to the classical replicative mechanism involving Shapiro intermediate and a resolution step, this pathway is characterized by the formation of a single-stranded circular DNA intermediate as a product of a strand-transfer reaction between terminal inverted repeats (TIRs) of the transposon as a very first step. The circular DNA thus carries a junction of TIRs with a few base pairs long linker originating from a transposon flanking sequence. The DNA circle is then repaired by host factors and integrated in a targeted or random manner as a second step. Both steps depend on a poorly characterized transposase featuring a DDE type of catalytic domain with a high potential for mobilization of various genetic elements including antibiotic resistance genes and different promoters. We determined X-ray structures of a dimeric transposase *from ISCth4* transposon from *C. thermocellum* in a complex with DNA intermediates in the copy-out- paste-in pathway. The structures show how the transposase recognizes its TIRs in a bipartite manner and suggest conformational changes that control the position of DNA flanking sequences in the active site.

P33

PROTEIN PRODUCTION IN MAMMALIAN CELLS VS *E. COLI* – IMPACT ON Tau PROTEIN PROPERTIES

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Hyperphosphorylated and abnormally post-translationally modified Tau protein forms aggregates called neurofibrillary tangles (NFTs), which are typical hallmark of Alzheimer's disease (AD) in the human brain [1]. As the pattern of post-translational modifications (PTMs) within Tau is complex [2], AD-relevant Tau protein is not easily obtainable by recombinant production in *E. coli* and subsequent modification by individual enzymes *in vitro*. To prepare Tau protein with naturally occurring PTMs, we employed mammalian cell culture (HEK293). We aimed to identify the PTMs incorporated in the HEK293 cells, to compare them with published data from AD-patients and afterwards to study the impact of the PTMs on Tau properties in comparison to Tau from *E. coli*.

We optimized the expression and purification protocols yielding sufficient amount of HEK-Tau for its characterization. Using LC-MS/MS, we identified around 20 phosphorylation sites with diverse extent of phosphorylation. The detected phosphorylation patterns were similar to those found in the brains of AD-patients. Afterwards, we performed interaction study between HEK-Tau and 14-3-3ζ protein, which provided surprising result compared to Tau expressed in *E. coli* and phosphorylated *in vitro* [3]. Finally, we used Thioflavin T assay and negative stain EM to obtain insight into aggregation propensities of HEK-Tau.

The protein production in HEK293 cells was performed within an Industrial PhD programme in the biotech company BioVendor.

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This project has received funding from the European Union's Horizon Europe program under the grant agreement No. 101087124. We acknowledge CEITEC Proteomics Core Facility of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042, CZ.02.01.01/00/23_015/0008175, e-INFRA CZ (ID:90254)). We acknowledge Cryo-electron microscopy and tomography core facility CEITEC MU of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042) and European Regional Development Fund-Project „Innovation of Czech Infrastructure for Integrative Structural Biology"(No. CZ.02.01.01/00/23_015/0008175).

P34

STRUCTURAL CHARACTERISATION OF PROTEINS INVOLVED IN THE METABOLISM OF R-LOOPS

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During transcription, RNA polymerases (RNAP) may pause, creating obstacles on the DNA, which increase the risk of collision with other DNA-centred processes such as DNA replication. If these conflicts are left unresolved, they may result in replication fork stalling and subsequent double-strand DNA breaks. Pausing of RNAP may also lead to the formation of R-loops – tripartite nucleic acid structures in which nascent RNA hybridises with its complementary DNA strand, leaving the non-template strand unpaired. Persistent R-loops may be responsible for DNA damage due to the exposure of fragile single-strand DNA.

Helicase senataxin (SETX) is a member of the SF1B-family of helicases, which, in contrast to its yeast counterpart – Sen1 – translocates preferentially on RNA [1]. Fittingly, SETX's preferred substrate is an R-loop [1]. Additionally, SETX terminates transcription in a species-specific manner [1], suggesting that specific, hitherto unknown, sequence features have evolved to accommodate

this feature. These properties of SETX place it as a transcription termination factor specialised in R-loop metabolism and control, which is essential for the resolution of transcription-replication collisions, crucial for the maintenance of genome stability. Since the fundamental functional differences between SETX and Sen1 are likely based on distinct structural features and no experimental structural data is yet available on SETX, we resort to methods of structural biology to mechanistically investigate the functions of SETX.

In our poster, we present our latest results, which bring first insights into the molecular mechanism(s) behind the functions of SETX.

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P35

STRUCTURAL INSIGHTS INTO FATTY ACYL DESATURASES AND REDUCTASES IN INSECT PHEROMONE BIOSYNTHESIS

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Fatty acyl desaturases (FADs) and reductases (FARs) are key enzymes in insect pheromone biosynthesis, catalyzing the modification of fatty acyl-CoAs into species-specific pheromone precursors or components. The specificity of these enzymes is determined by structural features within the substrate cavity, which influence substrate binding and catalysis. Understanding these structural features is essential for elucidating the molecular basis of pheromone divergence, and for engineering enzymes with tailored specificities.

To identify residues modulating enzyme specificity, we use AlphaFold 2 and 3 to predict FAD and FAR models. Such models facilitate mutant design, enabling us to probe the functional role of specific residues in substrate recognition and regiospecificity. Engineered enzymes are expressed in yeast, followed by lipid analysis using gas chromatography. This approach has revealed critical residues that influence chain-length specificity and regiospecificity without impairing catalytic activity.

Using structure prediction, we can efficiently identify candidates with selected specific characteristics. Our findings provide a framework for accelerating specificity prediction and engineering enzymes to tailor product properties. This approach has broad applications in biotechnology, e.g., pheromone-based pest control.

P36

STRUCTURE OF Tau FILAMENT CORE FRAGMENT dGAE WITH MN423 ANTIBODY

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Intrinsically disordered proteins, tau protein being our focus, lack stable tertiary structure, but form a conformational ensemble of molecular states exhibiting dynamic and complex network of intramolecular interactions. In a group of human diseases called tauopathies tau protein aggregates and propagates the pathology. To uncover specific conformation of tau aggregates, we performed X-ray crystallography investigation of the tau filament core fragment dGAE (tau297-391) complexed with filament conformation specific antibody MN423.

Tau fragment and antibody Fab were prepared by recombinant expression in *E.coli* and CHO cell line, respectively, purified to homogeneity and the complex was isolated by size exclusion chromatography of the mixture of both proteins. Crystals of complex were obtained from hanging drops, fished out with nylon loop and flash-cooled in liquid nitrogen. Diffraction data were collected at beamline PXI (SLS, PSI, Villigen, Switzerland). Diffractions were indexed in *P*1, integrated and scaled in XDS package and data were merged in TRUNCATE [1-3]. Diffractions were indexed in *P*1, integrated and scaled in XDS package and data were merged in TRUNCATE [1-3].

Molecular replacement was employed to solve the phase problem using the structure of MN423 Fab as a model (PDB ID 2v17). Asymmetric unit of crystal contained six molecules of Fab; we used variable and constant domain of antibody Fab separately as models to overcome the possible difference in elbow angles in the model molecule. Using REFMAC/COOT for macromolecular refinement we further refined the structure. Despite the

satisfactory fit of the model into electron density, we are not able to lower substantially R-factors (Figure 1).

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This work was supported by the H2020-MSCA-RISE-2019 Grant number 873127. This work was also funded by the grants APVV 21-0479, Vega 2/0125/23 and 2/0141/23.

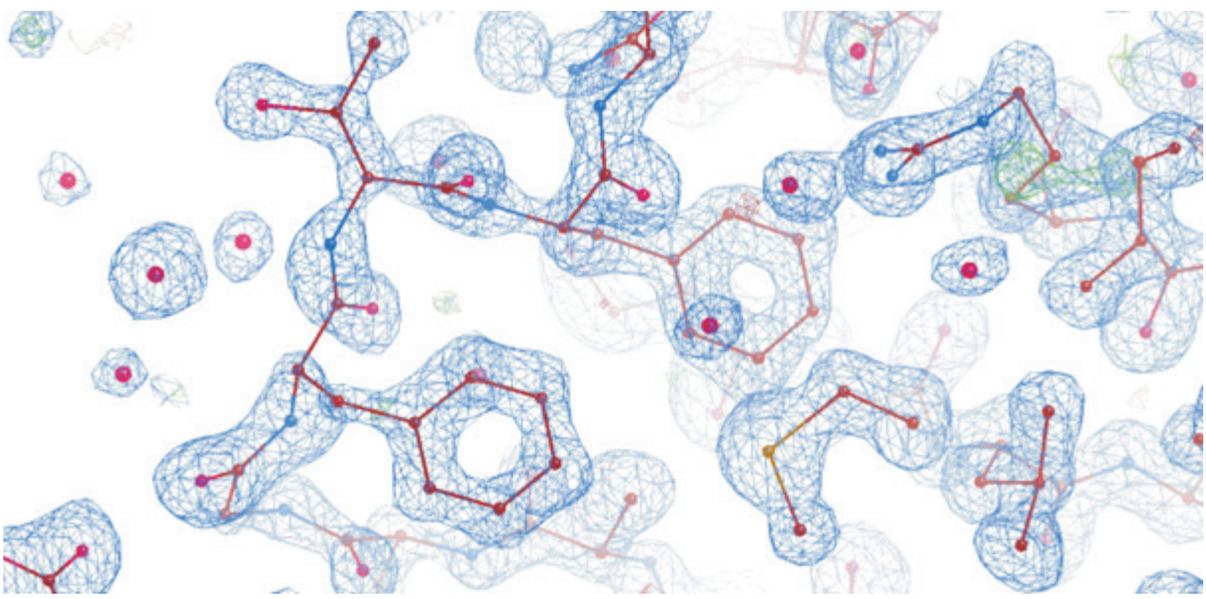

Figure 1. Modelled structure fit to electron density. 2Fo-Fc electron density map (blue) is contoured at 1,5 sigma.

P37

DISCOVERING STRUCTURAL SECRETS OF A JACALIN-RELATED LECTIN: A FUN GUY AMONG THE FUNGI

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This study focuses on the investigation of the properties and structural analysis of the lectin, a saccharide binding protein, derived from the mushroom *Calocera viscosa* (CalVL). Mushroom lectins have been extensively studied over the decades for their potential applications in biomedicine and diagnostics. Through their glycan-binding abilities, these lectins demonstrate significant biological activities, including antiproliferative, antimicrobial and mitogenic effects [1].

Lectin CalVL was determined to adopt a β-prism I fold, a structural characteristic shared by all the members of the jacalin-related lectins (JRLs) family. JRLs are typically divided into two groups based on their saccharide preference: gJRLs and mJRLs, preferring D-galactose and D-mannose, respectively [2].

CalVL was produced in the *Escherichia coli* expression system and subsequently purified through affinity chromatography using a mannose-agarose resin. Agglutination assays demonstrated that CalVL can agglutinate both yeast cells and human erythrocytes due to the interaction with their surface saccharides. Further analysis of binding properties by a glycan array resulted in narrow range of biantennary complex *N*-glycans which are likely its optimal binding partner. CalVL was successfully crystallized

in various conditions using vapour diffusion method, particularly the sitting drop technique. X-ray diffraction data were collected at the synchrotrons PETRA III in Hamburg and BESSY II in Berlin, Germany. Preliminary structures of CalVL in apoform, with D-mannose and *N*-acetylglucosamine are nearing the completion of the refinement process. The phase problem was solved through molecular replacement, employing a CalVL model predicted by AlphaFold2. To the best of our knowledge, CalVL represents the first structurally characterized fungal JRL among the animal and plant members of this family.

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CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project „Innovation of Czech Infrastructure for Integrative Structural Biology“ (No. CZ.02.01.01/00/23_015/0008175), is gratefully acknowledged for the financial support of the measurements at the CF Biomolecular Interactions and Crystallography.

P38

STRUCTURES OF THE GCPII AND PSMA-617 AND ITS DERIVATIVES WITH MODIFIED LIPOPHILIC LINKER REGIONS COMPLEXES

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PSMA-617 is widely recognized as a benchmark ligand for prostate-specific membrane antigen (PSMA) due to its extensive use in prostate cancer (PCa) targeted radionuclide therapy. This poster explores the structure of PSMA-617 alongside two novel analogs with modified linker regions. In compounds P17 and P18, the 2-naphthyl-L-Ala moiety was substituted with a less lipophilic 3-styryl-L-Ala moiety, while P18 also features a phenyl group in place of the cyclohexyl ring. The first-ever crystal structure of the PSMA/PSMA-617 complex reported here revealed a folded conformation of the PSMA-617 linker. In contrast, the PSMA/P17 and PSMA/P18 complexes exhibited extended linker orientations, demonstrating linker flexibility within the PSMA cavity – an insight that can be leveraged for structure-guided design of PSMA-targeting agents. Despite their structural modifications, the analogs retained strong PSMA inhibition potency, cellular binding, and internalization. These findings, together with the structure–activity relationships and *in vivo* biodistribution studies discussed in the manuscript under review, provide a strategic framework for the rational design of PSMA ligands, paving the way for the development of next-generation theranostic agents for PCa.

P39

ROLE OF NON-CANONICAL NUCLEOTIDES IN PROTORIBOSOME

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Our group has a long-standing interest in the molecular details of protein synthesis. This key cellular process occurs within large protein-RNA complexes called ribosomes. The part of the ribosome that surrounds the peptidyl transferase center (PTC), which is responsible for peptide bond formation, is known as the protoribosome [1]. Many of the details about the role of non-canonical nucleotides in the protoribosome remain unknown. Molecular dynamics simulations, using the software GROMACS were used to study differences in the behaviour of protoribosome with non-canonical bases and protoribosome with non-canonical bases replaced by canonical ones. This work may shed

some light on the reasons why non-canonical bases remain in the ribosome and are not replaced by canonical bases.

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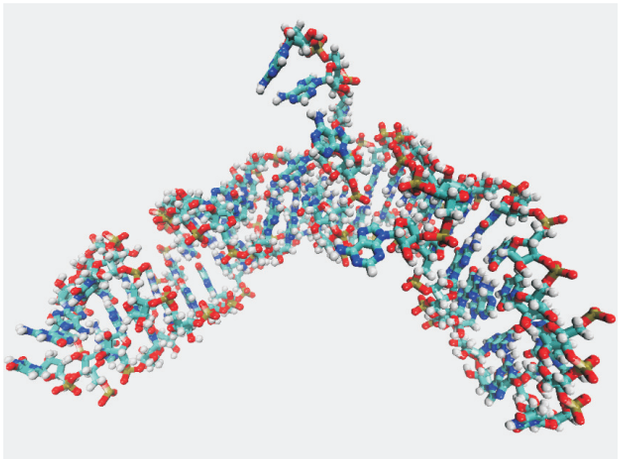

Figure 1. Model of protoribosome

P40

CORE FACILITY DEVOTED TO CRYSTALLIZATION OF PROTEINS AND NUCLEIC ACIDS, CENTRE OF MOLECULAR STRUCTURE, IBT CAS

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The Centre of Molecular Structure at IBT CAS (BIOCEV, Vestec, Czech Republic) is a sophisticated completels with comprehensive imaging capabilities.

A notable technological advancement is the SONICC instrument (Formulatrix) integrated with the RI1000 crystallization hotel. This system employs Second Harmonic Generation (SHG) and Ultraviolet Two-Photon Excited Fluorescence (UV-TPEF) techniques, enabling unprecedented detection of micro- and nanocrystals. Such precise screening is crucial for advanced methodologies, allowing researchers to analyze crystallization experiments with exceptional sensitivity and precision. The software environment of crystallization hotels is currently undergoing a major upgrade to ensure long-term sustainability and autox of scientific core facilities specializing in structural biology. Its crystallization core facility uses among other equipment also automated robotic systems for high-throughput crystallization experiments and specialized crystallization homated scoring using multiple evaluation algorithms.

The facility's equipment represents cutting-edge technology in structural biology research, facilitating complex crystallization experiments and detailed structural analysis of biomacromolecular complexes.

P41

VALIDATION OF SMALL STRUCTURE MOTIFS IN DISORDERED PROTEINS BY MOLECULAR DYNAMICS SIMULATIONS

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Local structure motifs in the shape of monocyclic or polycyclic, low-membered rings stabilized by main chain-main chain or main chain-side chain hydrogen bonds are frequent in globular proteins. They often contribute to the formation of helical or flat ribbon structures. It has been proposed that these ring structures may exist at least transiently also in intrinsically disordered proteins (IDPs) intimately imprinting their conformational ensemble [1]. Our hypothesis is that these motifs are determinants of physiological and pathophysiological properties of IDPs. The implication of these motifs for IDP tau is in the process of amyloid formation during tau aggregation. Amyloids are characterized by highly ordered cross-β structures and we suppose the role of these motifs in regulation of misfolding and aggregation. Interestingly, several motifs can be identified on existing crystal structures of tau protein complexes [1, 2]. To investigate the effect of these motifs on tau protein, we can use site-directed mutagenesis following the changes of tau aggregation *in vitro* and alteration of affinity to tau interaction partners. Another tool for characteriza-

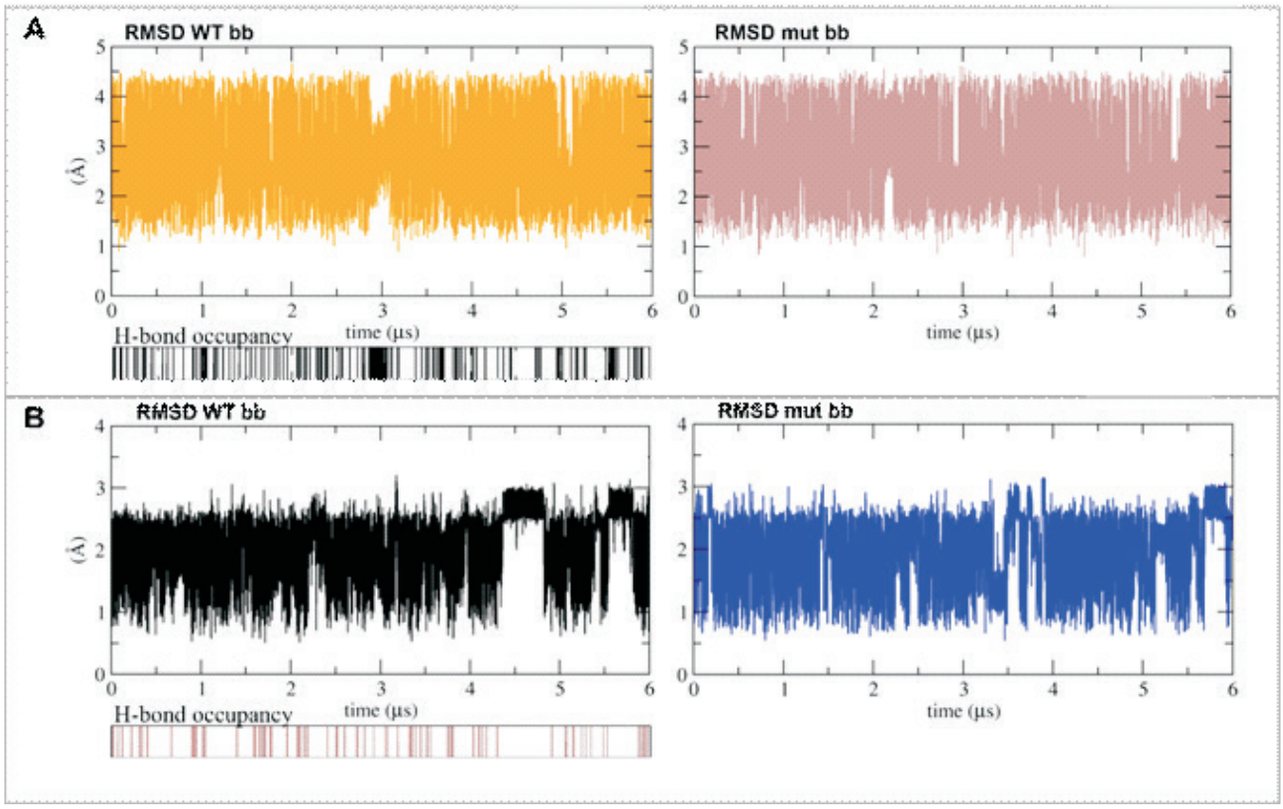

Figure 1. Molecular dynamics of tau peptides. (A) 298KHVPGGGSVQIVYK311, (B) 382AKAKTDHGAEIVY394. Both peptides were simulated as WT (left) and with mutated residue creating small motif in WT (right) revealing the influence of small motif for the conformational ensemble of disordered peptide. RMSD analysis were performed against backbone atoms of X-ray structures involving the peptide – 5MO3 in (A) and 2V17 in (B).

tion of these small structural motifs are molecular dynamics methods (MD).

We used MD to observe the formation of the motifs on tau fragments in solution – without the constraint of interaction partners as for the crystal structures. We simulated three fragments encompassing following two motifs identified on tau protein: β-*turn* formed by interaction of main-chain carbonyl of Gly302 with main-chain amide of Ser305 in the peptide tau298-311 and *Asx-turn* formed by interaction of side chain carboxyl of Asp387 with main chain amide of Gly389 in two peptides – tau381-391 and tau382-394. We validated the existence of the motifs based on H-bond analysis. We also compared the effect of these motifs on conformation ensemble by mutation of Ser305Ala for the first peptide and Asp387Ala for the second and third peptide. The β-turn induced formation of metastable populations that were stabilized by the motifs and in accordance to low radius of gyration values as these conformations were at least partially folded. For Asx-turn and 382-394 fragment, both WT and mut fragments adopted hairpin conformation stabilized by salt bridges. Interestingly, even though the Asx-turn was not highly populated and no major effect was observed on formation of metastable populations, the hairpin conformation was shifted towards C-terminus for the mutant fragment. Our next steps consist of *in-vitro* part – to biophysically characterize these short peptides and then to assess the effect of mutations of selected motifs on aggregation of tau filament core fragment tau297-391 (dGAE).

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This work was supported by the European Union's Horizon Europe program under the grant agreement No. 101087124. This work was also funded by the grants APVV 21-0479, Vega 2/0125/23 and 2/0141/23. Part of the research results was obtained using the computational resources procured in the national project National competence centre for high performance computing (project code: 311070AKF2) funded by European Regional Development Fund, EU Structural Funds Informatization of society, Operational Program Integrated Infrastructure

P42

LASER-DRIVEN PLASMA X-RAY SOURCE AT ELI BEAMLINES

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ELI Beamlines

We report on experimentally measured characteristics of a kHz laser-driven Cu plasma X-ray source that was recently commissioned at ELI Beamlines facility. The source is powered by a 50-fs TW laser, producing X-ray pulses that enable sub-ps resolution for time-resolved experiments. The X-ray source parameters with the two driving lasers are compared, providing photon flux up to the order of 10^{13} photons/4π/s. Besides the X-ray beam characteristics, the experimental platforms for ultrafast X-ray diffraction and X-ray absorption and emission spectroscopy are described to outline the possible application experiments, as the system will be operated in a user-based access mode.

P43

PhiKZ BASEPLATE STRUCTURE

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

P44

IMPROVED VALIDATION AND REFINEMENT OF BIOMOLECULAR STRUCTURES

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We present a further development of datasets and methods available in our laboratory for improved validation and refinement of biomolecular structures. We are building on synergies of data and tools involving structural alphabets [1-3] and flexibility of biomolecules [4,5].

The conformation- and location-dependent flexibility and solvation behavior derived from high quality non-redundant biomolecular structural data was employed for improvement of protocols for validation and refinement of biomolecular structures.

We extracted normalized B factor values of protein residues derived from high resolution structural data and collected distributions for protein residues in the interior and residues at the surface, either not found in contacts or involved in direct or water mediated contacts with biomolecular binding partners.

For structure validation we compared how well the B factor distribution in a model follows the corresponding reference distribution. The expected reference distribution from high-resolution structures can be also used prior to structure refinement replacing the currently used uniform B factor values. This can improve the convergence as well as accuracy of the structure refinement.

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P45

FUNCTIONAL CHARACTERISATION OF THE LUMINOUS APPARATUS OF THE SEA PEN *PENNATULA PHOSPHOREA*

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Bioluminescence is the production of visible light by living organisms. It occurs through the oxidation of specific luciferin substrates catalysed by luciferase enzymes. Auxiliary proteins, such as fluorescent proteins and coelenterazine-binding proteins, can modulate the wavelength of emitted light or stabilise reactive luciferin molecules. Bioluminescent organisms offer a variety of light-emitting enzymes and photoproteins with immense potential for bioengineering applications, from biosensors to zero-electricity lighting solutions. However, despite the abundance of marine bioluminescent species, only a few systems have been biochemically and structurally characterised. Among anthozoans, the *Renilla*-type bioluminescence is the most studied [1,2], relying on a coelenterazine -dependent luciferase, a calcium-dependent coelenterazine- binding protein, and a green fluorescent protein.

Recently, transcriptomic analyses identified putative luciferase and auxiliary protein sequences responsible for bioluminescence in the sea pen *Pennatula phosphorea* [3]. Here, we perform biochemical reconstitution and functional characterisation of the *Pennatula*-encoded luciferase, providing insight into its light-emitting properties.

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This work was supported by the Czech Science Foundation (GX25-17329X) and by the European Union Centre of Excellence CLARA (101136607). M.S. and D.P. are Brno Ph.D. Talent Scholarship holders funded by the Brno City Municipality.

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CF DIFFRACTION TECHNIQUES IN CENTRE OF MOLECULAR STRUCTURE: EMPLOYING HIGH-END X-RAY TECHNOLOGIES FOR LABORATORY STRUCTURAL BIOLOGY

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The Centre of Molecular Structure (CMS) provides services and access to state-of-art instruments, which cover a wide range of techniques required by not only structural biologists. CMS operates as part of the Czech Infrastructure for Integrative Structural Biology (CIISB), and European infrastructures Instruct-ERIC and MOSBRI. CMS is organized in 5 core facilities: CF Protein Production, CF Biophysics, CF Crystallization of Proteins and Nucleic Acids, CF Diffraction Techniques, and CF Structural Mass Spectrometry.

CF Diffraction Techniques employs two laboratory X-ray instruments equipped with high flux MetalJet X-ray sources: a single crystal diffractometer D8 Venture (Bruker) and a small angle X-ray scattering instrument SAXSpoint 2.0 (Anton Paar). The configurations of both instruments represent the top tier of possibilities of laboratory instrumentation. Apart from standard applications, the instruments are also extended for advanced experiments: the diffractometer is equipped with the stage for in-situ crystal diffraction, X-ray flourscence detector and crystal dehydration, SAXS is equipped with in-situ UV-Vis spectroscopy and a liquid chromatography system for SEC-SAXS. The setups enable easy access and fast turn-around of samples under different conditions, but also collection of high quality end-state data without further need for synchrotron data collection in many cases. CF Diffraction Techniques provides services in synergy with the other CFs on-site, therefore scientific questions can be quickly answered as they emerge from the experiments.

The Centre of Molecular Structure is supported by: MEYS CR (LM2023042); project Czech Infrastructure for Integrative Structural Biology for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF; UP CIISB (CZ.02.1.01/0.0/0.0/18_046/0015974), ELIBIO (CZ.02.1.01/0.0/0.0/15_003/0000447), and MOSBRI from EU Horizon 2020 (No. 101004806).

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MAVISp: A MODULAR STRUCTURE-BASED FRAMEWORK FOR PROTEIN VARIANT EFFECTS

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The role of genomic variants in diseases, including cancer, continues to expand thanks to the advent of advanced sequencing techniques integrated into clinical practice [1]. The rapid growth in the identification of genomic variants has led to the classification of many variants as Variants of Uncertain Significance (VUS) or those with conflicting evidence, posing challenges in their interpretation and application. Additionally, current methods for predicting pathogenic variants do not necessarily provide information on the mechanisms underlying pathogenicity [2-4]. MAVISp (Multi-layered Assessment of VarIants by Structure for proteins), a modular structural framework for variant effects, to handle high-throughput saturation variant analysis with a standardised workflow, integrating results with various pathogenicity predictors [5]. Currently, MAVISp offers analyses for 500 different proteins, encompassing more than 3 million variants. The framework facilitates the analysis of variant effects at the protein level and has the potential to advance the understanding and application of mutational data in disease research. In this context, we propose an additional module based on an Adversarial Autoencoder [6] to enhance the systematic understanding of mutational effects and to establish a foundation for further investigations that remain unexplored, thereby broadening the scope of future research in this domain. The application was carried out in the case study of p53.

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CRYSTAL STRUCTURE OF HUMAN NK CELL ACTIVATION RECEPTOR NKp80

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Natural Killer (NK) cells, a subset of effector lymphocytes, can spontaneously destroy target cells, such as infected, damaged, or malignant cells. NK cell cytotoxicity is mediated by activating receptors on their surface, such as NKp80 (Natural Killer protein 80 kDa) [1]. The human receptor NKp80 stimulates cytotoxicity through its interaction with its ligand AICL (Activation Induced C-type Lectin), which is constitutively expressed by all myeloid cells. In pathological conditions, such as cancerous or damaged cells, AICL is often upregulated, resulting in the lysis of these cells by NK cells expressing NKp80 [2]. This interaction is thus a promising immunotherapeutic target for treating myeloid leukaemia.

However, the structures of both proteins have remained elusive. Hence, we have focused on successfully producing the extracellular domain of NKp80 in sufficient quality and quantity. Here, we introduced a series of mutations in the stalk region to study their effect on the production, stability, and homodimer formation. Using stably transfected HEK293S GnTI$^-$ cells, we produced in total seven variants of NKp80, replacing cysteines with serines, and the proteins were analysed using techniques such as size-exclusion chromatography, differential scanning fluorimetry, and mass spectrometry.

Our findings demonstrate a substantial improvement in the production yield for six of the seven NKp80 mutants, with some variants exhibiting up to a fivefold increase compared to the wild-type NKp80 extracellular domain. Consequently, we have selected the two most successful variants for large-scale production to enable crystallization trials. The trials resulted in the elucidation of the hitherto unknown structure of NKp80 homodimer at a resolution of 2.9 Å. The NKp80 homodimer adopts an overall conformation similar to that of the homodimer of human NKR-P1 [3], with helices α1 forming the dimerisation interface. The stalk region included in the expression construct was not observed in the crystal structure; in fact, the only NKp80 construct/preparation to crystallize was the one that showed limited proteolysis upon enzymatic deglycosylation resulting in a loss of most of the stalk region, as confirmed by N-terminal protein sequencing. Thus, the NKp80 stalk is probably unstructured or very flexible, at least when the extracellular part of NKp80 is produced in a soluble form and is not tethered to the transmembrane part and the rest of the whole receptor.

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This research was funded by the Czech Science Foundation (25-18490S), the Ministry of Education, Youth, and Sports of the Czech Republic (LUAUS25250), and Charles University (GAUK 318122). K. B. received STSM support from the COST Action CA18103 INNOGLY. The authors also acknowledge the support and the use of resources of the Instruct-ERIC infrastructure.

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NMR STRUCTURAL AND INTERACTIONAL CHARACTERIZATION OF HUMAN Tau PROTEIN

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Tau protein is a microtubule-associated protein localized and expressed in human brain cells, predominantly in axons of neurons. The function of this protein is imperative for correct axonal microtubule formation and function. Tau protein has been found to form aggregates and filamentous structures in the brains of patients suffering from Alzheimer's disease or other neurodegenerative diseases. However, the exact molecular mechanism of neurodegeneration remains unknown. Tau protein belongs to the group of intrinsically disordered proteins, which makes NMR a valuable tool in analysis of its structural properties and interactions [1, 2].

In this project, several advanced NMR techniques were utilized to study the properties of Tau protein. Firstly, temperature effects on secondary structure propensities of full-length tau protein (tau isoform 2N4R) were studied. Secondly, we studied the effect of phosphorylation by GSK-3 beta kinase on the interaction of Tau protein with microtubules. Additionally, the expression of Tau protein in E. coli cells was carried out, with the intention to optimise the expression protocol for effective incorporation of fluorinated amino acids, with further goals to use this construct for in-cell NMR measurements.

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This project has received funding from the European Union's Horizon Europe program under the grant agreement No. 101087124 – ADDIT-CE and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 873127 – InterTAU. We acknowledge CF Prot and CF NMR of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042) and European Regional Development Fund-Project „UP CIISB" (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

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STRUCTURE AND DYNAMIC PROPERTIES OF PORPHYRIN AGGREGATES IN SOLUTION

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Multiple factors influence the aggregation behavior of porphyrins in solution; however, obtaining detailed structural and dynamic insights remains challenging. Motivated by intriguing yet puzzling experimental data from UV/VIS and NMR spectroscopies, we investigated the aggregation of 3,4,5-TEG-TPP porphyrins. We analyzed the stability and dynamic properties of these molecules in solution using various computational methods. We developed detailed models of solvated porphyrin monomers, dimers, and larger aggregates, which underwent relaxation before being propagated through molecular dynamics simulations. From the sampled trajectories, we computed optical and magnetic spectra and compared them with experimental results. This approach provides valuable insights into the mechanisms of porphyrin aggregation in aqueous envi-

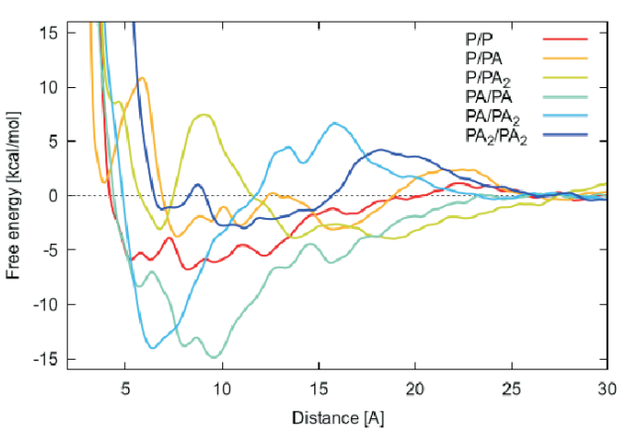

Figure 1. Free energy profile of dimers with different charge.

ronments and enhances our understanding of their structural stability.

Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by the Ministry of Education, Youth and Sports of the Czech Republic.

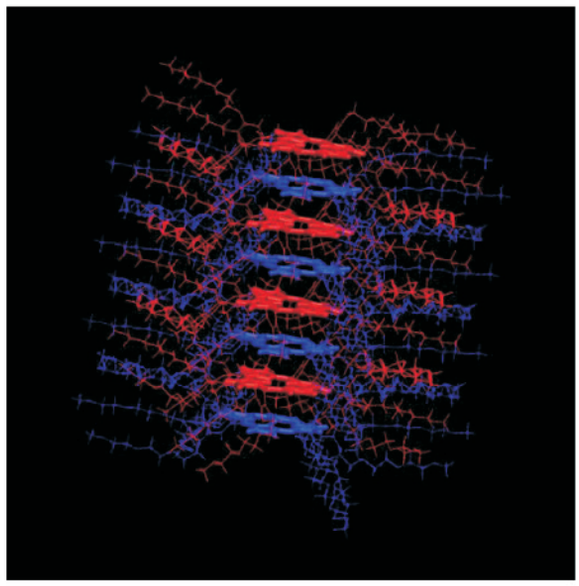

Figure 2. Model of aggregate.

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A pY READER OF ARID1A FACILITATES SIGNAL-DEPENDENT cBAF ACTIVITY

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ARID1A, a key component of the cBAF chromatin-remodelling complex, is essential for maintaining cBAF functionality and structural integrity [1].

Here, we reveal that specific phosphotyrosine (pY) residues within the intrinsically disordered region (IDR) of ARID1A play a pivotal role in modulating its function and localization.

Using a combination of NMR spectroscopy, mass spectrometry and confocal fluorescence microscopy, we identify a novel pY reader specific to these phosphotyrosines, which interacts with ARID1A in a phosphorylation-dependent manner. Importantly, phosphorylation of these tyrosine residues alters the cellular distribution of the pY reader, relocalizing it to specialized nuclear compartments. This regulatory mechanism underscores a phosphorylation-sensitive interaction network, potentially controlling ARID1A's recruitment and activity within chromatin remodelling processes. These findings highlight a new layer of regulatory complexity in cBAF-mediated chromatin dynamics and suggest a broader role for post-translational modifications in directing subnuclear compartmentalization.

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Technická redakce: I. Kutá Smatanová, R. Kužel
ISSN 1211 – 5894 díl **31** (2025), číslo 1 CSCA

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XXI Discussions in Structural Molecular Biology and the 8th User Meeting of CIISB are supported by the CIISB research infrastructure project LM2023042 of the Ministry of Education, Youth, and Sports of the Czech Republic, and companies NanoTemper, Specion, Pharmatech, BioRad, Biotech, Merck, Anamet, Cytiva and APCzech.