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# POSTERS

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Abstracts submitted to the 49th FEBS Congress from 5th to 9th July 2025 and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. The abstracts are available as two PDF files: Talks (Plenary Lectures, Symposia and Special Sessions) and Posters.

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### How to cite these abstracts

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* Each poster has been given a unique number beginning with the letter P (or LB for 'late-breaking abstracts'); the next numerical part relates to the topic grouping as listed below.



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acetylmuramoyl-L-alanine amidase activities, targeting cross-linked tetrapeptide structures within the cortex. Here, we present the crystal structure of intact SleC, including its pre-domain, pro-domain, and hydrolase domain, in a tetrameric form at 2.17 Å resolution.

## P-18-030
## Algorithm-driven optimization of cysteine protease mutagenesis: GROMACS-based mutagen selection for enhanced solubility

**M. Dabiri**, Z. Levarski, J. Turna, S. Stuchlík, E. Struharnanska
*Faculty of Natural Sciences, Comenius University, Bratislava, Ilkovicova 3278/6, 841 04 Karlova Ves, Bratislava, Slovakia*

Cysteine protease (CysPC) DEK1 is a key regulator of plant development, but its poor solubility limits structural and functional studies. To address this, we developed a reinforcement learning-based computational framework integrating molecular dynamics (MD) simulations via Groningen MAchine for Chemical Simulation (GROMACS) with systematic mutagen selection to optimize DEK1 solubility while preserving its structural stability. Our approach employs Proximal Policy Optimization (PPO), a reinforcement learning algorithm that iteratively explores mutation combinations based on solubility-related physicochemical properties. Key input features extracted from GROMACS simulations include hydration free energy, radius of gyration, solvent-accessible surface area (SASA), secondary structure stability, and interaction energy with water molecules. These features guide the reinforcement learning model in selecting beneficial mutations that enhance solubility. The PPO-based strategy enables adaptive learning, allowing the model to refine mutation predictions dynamically. Validation through *in silico* free energy calculations and comparative molecular dynamics simulations confirms that PPO-driven mutagenesis selection improves solubility more effectively than heuristic-based approaches. This study introduces a data-driven, adaptive framework for protein solubility optimization, with potential applications in protein engineering, drug development, and structural biology. The integration of reinforcement learning with molecular dynamics paves the way for more efficient and scalable protein design methodologies.

## P-18-031
## Structural insights into regulation of apoptosis signal regulating kinase 1 (ASK1) by 14-3-3 protein

**J. B. Bhosale**[I,II], D. Košek[I], V. Obšilová[I], T. Obšil[I,III]
[I]*Department of Structural Biology of Signaling Proteins, Division BIOCEV, Institute of Physiology of the Czech Academy of Sciences, Vestec, Prague, Czech Republic,* [II]*Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic,* [III]*Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Prague, Czech Republic*

ASK1, also known as MAP3K5, is a crucial stress sensor and upstream regulator of p38 and JNK signaling pathways. Dysregulation of ASK1 has been seen in cancer, inflammatory, cardiovascular and neurodegenerative diseases. Hence, ASK1 activity has to be strictly regulated to respond stress stimuli appropriately. In unstressed conditions, ASK1 is held in an inactive state termed as signalosome through binding of negative regulators, thioredoxin (TRX1) and 14-3-3. Recently, a cryo-EM structure of ASK1 revealed its dimeric and asymmetric architecture, suggesting possible ways to interact with 14-3-3. It is a highly conserved phosphoserine/phosphothreonine-binding scaffold protein and, 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. This project aims to unravel the structural and molecular basis of ASK1 regulation by 14-3-3 protein. The SV-AUC experiments revealed that ASK1 and 14-3-3 form a complex with apparent dissociation constant ($K_D$) of □90 nM. The sedimentation coefficient distribution c(s) also suggested that the presence of 14-3-3 induced the tetramerization of ASK1, i.e. a complex with a stoichiometry of 4:4 (two ASK1 dimers forming a tetramer stabilized by two 14-3-3 dimers). The formation of a 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, presumably also interactions between the kinase domains and MAP2K kinase substrate, thus explaining the inhibitory effect of 14-3-3 binding. Our results provide the first insight into the structural basis of 14-3-3 protein-mediated regulation of ASK1 kinase.

## P-18-032
## Deciphering the interaction of botulinum neurotoxin A light chain (LCA) with human septins

E. C. Mamani, I. Cavini, R. C. Garratt, **A. P. U. Araujo**
*Institute of Physics of São Carlos, University of São Paulo, São Carlos, Brazil*

Septins are a large family of proteins that bind to guanine nucleotides and interact with each other to form cytoskeletal filaments, which can organize into higher-order structures. These filaments are considered the biologically active form of septins and play crucial roles in several cellular processes. Consequently, the absence or dysfunction of these proteins is associated with various pathologies. Previous studies on septins have revealed their ability to interact with proteins from other families, expanding the scope of investigation into their biochemical and functional significance. One such protein is the light chain of botulinum neurotoxin type A (LCA), a metalloprotease capable of cleaving proteins essential for neurotransmitter release, ultimately leading to muscle paralysis. In this study, several experimental approaches were employed to investigate and characterize in detail the interaction between LCA and human septins. To this end, LCA and various septin complex constructs were expressed, purified, and used to evaluate interactions through pull down assays, microscale thermophoresis, and structural modeling. The results indicated that LCA interacts directly with septins 6 and 7, specifically at their C-terminal domains. Furthermore, mutations in specific LCA residues confirmed their importance for stable complex formation. Structural modeling enabled the prediction of the LCA C-terminal conformation in interaction with septins, allowing the identification and evaluation of other residues critical for this association and supporting the proposed model. In conclusion, this study advances the understanding of the LCA-septin interaction and proposes a plausible model for their association.


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