

The Biochemistry Global Summit

25th IUBMB Congress, 46th FEBS Congress, 15th PABMB Congress

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Lisbon, Portugal

Abstracts submitted to The Biochemistry Global Summit (25th IUBMB Congress, 46th FEBS Congress and 15th PABMB Congress) from 9th to 14th July 2022 and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this supplement. The abstracts are available as two PDF files: Talks (Plenary Lectures, Symposia and FEBS Special Sessions) and Posters.

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Abstracts submitted to the Congress are **not peer-reviewed**. In addition, abstracts are published as submitted and are **not copyedited** prior to publication.

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* The Abstract number can be found atop each abstract's title in the PDF file.

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Abstracts submitted to the virtual The Biochemistry Global Summit (25th IUBMB Congress, 46th FEBS Congress and 15th PABMB Congress) from 9th to 14th July 2022 and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this supplement. The abstracts are available as two PDF files: Talks (Plenary Lectures, Symposia and FEBS Special Sessions) and Posters.

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* Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented (see p.68 for key).

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isoforms largely determine the characteristics of the actin filament and may underlie the regulation of the interaction of actin with its partner proteins, which plays an important role in cancer cell transformation and metastasis. The work was supported by President grant MK-5708.2021.1.4.

P-04.1-090

Analysis of the structure and filament assembly of human septin complexes

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Septins are GTP binding proteins that are considered as a fourth element of the cytoskeleton. These proteins are capable of forming apolar filaments, composed of hetero-oligomeric complexes, which are important for cytokinesis and a variety of other cellular processes. The complexes present a specific subunit arrangement and the interaction between septins are made by two different interfaces, NC or G. However there is a variability in size and composition among species. In humans, these can be either hexameric or octameric complexes, in which two copies of three or four different septin groups, respectively, assemble into a specific order. The assembly mechanism and the polymerization of the septin complexes are still unknown. X-ray crystallography technique has been widely used to study the septin monomers or dimers, on the other hand, it's not feasible to obtain crystals and high resolution structures of the full septin heterocomplex. Recently, using Cryo-EM, it was possible to provide a complete model for a human septin hexameric complex, with a global resolution of ~3.6 Å. Previously published in: Mendonça DC et al. (2021) J Mol Biol 433, 167096. We observed a bending at the centre of the hexamer that can be related to their ability to interact with membranes. Furthermore we have better understood the NC interface between SEPT6 and SEPT7, where an internal cavity is present with a floor formed by two anchored α 0 helices including their polybasic regions. It's known that filaments can be organized in higher order structures in the cell. Our current work aims to explore the regions responsible for the interactions between paired filaments. These interfaces are believed to be stabilized by the formation of coiled-coils from the septin C-terminal domains. The optimization of the sample preparation is in progress and first cryo-EM analysis will be presented. Altogether, this should give us a more complete understanding of the septin structure-function relationship.

P-04.1-091

Comprehensive analysis of bactofilin in *Caulobacter crescentus*

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Similar to eukaryotes, bacteria utilize filamentous proteins and filament systems that are collectively known as "cytoskeletons"

to spatiotemporally organize cellular components. A widely conserved member of the bacterial cytoskeleton is the protein bactofilin. Bactofilin homologs are characterized by a conserved bactofilin domain that adopts a β -helical architecture and polymerizes spontaneously into large polymers such as bundles and 2D crystalline sheet (1). In the model organism *Caulobacter crescentus*, two bactofilin paralogues, BacA and BacB, form a sheet lining the stalked pole which recruits the peptidoglycan remodeling protein PbpC for stalk elongation (2). Using mutagenesis, subcellular localization studies and *in vitro* crosslinking, we identified several residues in BacA that are critical for its polymerization. It appears that the formation of protofilaments is mediated by hydrophobic amino acids at the end of bactofilin domain, whereas charged residues on the surface contribute to lateral interactions between protofilaments. Moreover, we confirmed that the membrane association of bactofilin is via a conserved N-terminal motif, as suggested by Deng et. al (3). A systematic mutational screening demonstrated that phenylalanine and lysine residues within this motif are indispensable for membrane binding. Finally, our study provides insight into the interaction between BacA and PbpC. Both *in vivo* and *in vitro* experiments suggest that the first 13 amino acids of PbpC are required for the interaction with BacA. We further narrowed down the PbpC-binding region of BacA by hydrogen deuterium exchange (HDX) analysis to the last winding of bactofilin domain. Together, our findings expand previous knowledge about bactofilin and shed new light on the mechanisms that underlie its biological function. References: 1. Vasa S et al. (2015) Proc Natl Acad Sci USA 112, E127–136. 2. Kühn J et al. (2010) EMBO J 29, 327–339. 3. Deng X et al. (2019) Nat Microbiol 4, 2357–2368.

P-04.1-092

Bringing *Ciona intestinalis* septins to light

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Septins are cytoskeleton proteins involved in many important cellular processes, such as cytokinesis, cell polarization and morphogenesis. Septins are ubiquitous in fungi and animals, and different subunits associate in the cell to assemble filaments, which build other sophisticated architectures. The number of genes coding for septins in different organisms is not homogeneous. *Ciona intestinalis* is a sea squirt largely used as a model system to explore the evolutionary origins of the chordate lineage and it possesses only four genes encoding septins. Interestingly, these genes represent one homologue for each group found in mammals: SEPT2, SEPT6, SEPT7 and SEPT9, predicting that *Ciona* septins are organized similarly to that observed in humans. Aiming to evaluate this, we produced and characterized the recombinant *C. intestinalis* septins (CiSEPT) and their complexes. Complexes containing three septins assemble as hexamers, but that with four septins (CiSEPT2-6-7-9) were non-homogeneous in length, showing the coexistence of hexamers and octamers. Using Transmission Electron Microscopy, CiSEPT2 was located at the end of the oligomers, agreeing with the positioning of the human SEPT2. Furthermore, we observed that the CiSEPT2-6-7-9 heterocomplex can assemble into higher order structures, similar to those already described for septins in other animals and yeast. Noteworthy, cryo-EM images show that, hexamers and octamers

can be clearly distinguished by the presence of two or three NC-interfaces, respectively. From the structural model fitted into the density map for the hexamer, the NC-interfaces can be seen to be involved in the formation of a cavity between subunits, similar to that described in the recent human structure. These results allow us propose *C. intestinalis* as a promising alternative model to study septins, satisfying the requirements for a complete but simpler model to understand the mechanisms behind the assembly of septin complexes and filaments.

P-04.1-093

Characterization of Kaiso binding sites in human kidney cancer cells

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DNA methylation is one of the key mechanisms involved in the regulation of transcription. Methylation of promoter regions often correlates with the inactivation of gene transcription. Methylated DNA attracts methyl-DNA binding proteins that are involved in transcriptional repression. Their depletion may result in gene activation and may serve as the basis of target therapy. Here, we investigated target genes of methyl DNA binding protein Kaiso in human kidney cancer cells. We performed ChIP-seq analyses for Kaiso binding sites and determined differentially expressed genes in Kaiso depleted cells obtained via CRISPR/CAS9 genome editing. We identified around 16000 regions bound by Kaiso. We confirmed that Kaiso may function as both transcriptional repressor and activator. Among up-regulated genes, we found PRKAR1B, RIOX1, SERPINF1, HSPB1, GABPB1-AS1, EML2 were downregulated. Also, we analyzed how the deficiency of Kaiso influences its target site's methylation. The methylation of the majority of Kaiso binding sites remained unchanged. However, we identified around 90 Kaiso binding sites in regulatory regions, that changed their methylation after Kaiso depletion. In most cases changes in DNA methylation did not lead to variation in the expression level of the target genes. Further, we introduced mutation on E535 amino acid in Kaiso that is abolished its ability to interact with methylated DNA but retained the ability to bind CTGCNA sequences. This mutation resulted in the loss of Kaiso's ability to bind to two-thirds of its binding sites. So, we can conclude that most of Kaiso DNA binding activity is related to interaction with methylated DNA. We identified new Kaiso target genes that can be activated or repressed by Kaiso. This research was funded by the Russian Science Foundation, project no. 19-74-30026 (Kaiso ChIP-seq), and by the Russian Foundation for Basic Research, № 19-29-04139 (E535 analyses).

P-04.1-094

Genetically controlled iron-oxide nanocompartments in mammalian cells based on encapsulin proteins

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We have demonstrated that spherical proteinaceous nanoreactors called encapsulins naturally occurring in bacteria such as *Quasibacillus thermotolerans* can be expressed in mammalian HEK293T cells. These encapsulins represent a two-component system consisting of a nanoshell and a natural ferroxidase cargo, enabling import and sequestration of up to 60000 iron atoms inside the nanocompartment. Genetic constructs and experimental details are given in Sigmund *et al.*, ACS Nano 2019, and Efremova *et al.*, Pharmaceutics 2021. We have identified that a small fraction of HEK293T cells grown in a Fe-containing medium and expressing these encapsulins can be isolated by commercially available columns for magnet-assisted cell sorting. Transmission electron microscopy and Energy Dispersive X-Ray Spectroscopy investigations revealed that each sorted cell contains thousands of electron-dense, Fe-containing nanoparticles with an average diameter of 30 ± 3 nm. Vibrating sample magnetometry proved the ferrimagnetic response of the sorted cells at 5-250K, which suggests the presence of magnetic phases different from antiferromagnetic ferrihydrite. Finally, we performed a proof-of-principle experiment showing the potential of encapsulin expression for cell manipulation when the cells were plated in the presence of a permanent magnet. According to the optical microscopy results, 24 hours later, the sorted cells were predominantly attracted to the edges of the magnetic cube immersed into the cell medium. The present study is the basis for a thorough understanding of the ferrimagnetism in mammalian cells via genetically encoded iron-storing proteins. We acknowledge support by the Federation of European Biochemical Societies (FEBS) via a Long-Term Fellowship, an Alexander von Humboldt Research Fellowship for Postdoctoral Researchers, an Add-on Fellowship for Interdisciplinary Life Science provided by the Joachim Herz Foundation (M.E.), as well as the Helmholtz RSF Joint Research Group (HRSF0064).

P-04.1-095

Preliminary identification of novel protein substrates for actin-histidine N-methyltransferase (SETD3)

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The SETD3 protein is widespread in multicellular eukaryotes and was recently identified as a protein histidine methyltransferase that catalyzes the N ϵ -methylation of histidine 73 residue in actin. This post-translational modification is important for maintaining cytoskeleton integrity and is the only well characterized