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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE AND FUNCTIONS OF THE TRANSCRIPTION AND TRANSLATION APPARATUS OF THE CELL

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Abstract P-29: Cryoem Study of the Inhibition of Bacterial Ribosomes by Madumycin II

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Background: The efficiency of widely used antibiotics is limited by continuous improvement of resistance mechanisms. Thus, the research of poorly studied drugs that have not received practical use until now becomes relevant again. Protein translation is one of the major targets for antibiotics. Madumycin II (MADU) is an antibiotic of the streptogramin A class that binds to the peptidyl transferase center of the initiated bacterial 70S ribosome inhibiting the first cycle of peptide bond formation (I.A. Osterman et al. *Nucleic Acids Res.*, 2017). The ability of MADU to interfere with translating ribosome is an open question that we address by investigation of high-resolution cryo-EM structures of MADU bound 70S ribosome complexes from *Escherichia coli*.
Methods: Purified initiated and translating ribosome complexes preincubated with MADU were applied onto freshly glow discharged carbon-coated grids (Quantifoil R 1.2/1.3) and flash-frozen in the liquid ethane pre-cooled by liquid nitrogen in the Vitrobot Mark IV. Frozen grids were transferred into an in-house Titan Krios microscope. Data were collected using EPU software. Movie stacks were preprocessed in Warp software. For image processing, we have used several software packages: Relion 3.1, CryoSPARC, and CisTEM. The model was built in Coot.
Results: We have obtained high-resolution cryo-EM structures of two ribosomal complexes with MADU before and after the first cycle of peptide bond formation with an average resolution of 2.3 Å. Preliminary analysis of the

structures shows no major differences in the MADU binding mode to the ribosomal complexes under study suggesting that the quantity of amino acid residues attached to the P-site tRNA does not impact MADU bonding. Moreover, in both cases, we observed similar destabilization of the CCA-ends of A- and P-site tRNAs underlining the comparable influence of MADU on the ribosomal complexes.

Conclusion: Our results suggest that although MADU binding site is located in the peptidyl transferase center, the presence of the second amino acid residue on the P-site tRNA does not preclude antibiotic binding. We assume that further elongation of the polypeptide chain would not have any impact either. High conformational lability of the CCA-ends of tRNA at the A and P sites upon binding of MADU obviously plays an important role in the inhibition mechanism of the bacterial ribosome. The further structural and biochemical analysis will be necessary to shed more light on the detailed mechanism of MADU action.

Key Words: ribosome • antibiotics • structure • cryo-EM

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