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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-14: Cryo-Electron Tomography Pipeline on the Example of Structural
Analysis of Polyribosomes**

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Background: Cryo-electron tomography (cryo-ET) is currently the only technique that can be used for quaternary structure determination of polyribosomal complexes under near physiological conditions. Here we describe, in more detail, the cryo-ET pipeline from a sample preparation for data processing from the poster talk “Cryo-ET structural analysis of polyribosomes from HeLa cells” by Zhanna A. Afonina.

Methods: The investigations were carried out in a Titan Krios 60-300 TEM/STEM (FEI, USA), equipped with Falcon II DED (FEI, USA) and C_s-image corrector (CEOS, Germany). Data acquisition were done automatically using FEI Tomography software. All data processing stages including sub-tomogram averaging after tomographic reconstruction using IMOD were carried out using computing resources of the Federal Collective Usage Center Complex for Simulation and Data Processing for Mega-Science Facilities at NRC “Kurchatov Institute”.

Results: Cryo-ET with sub-tomogram averaging was allowed us to determine spatial structure of polyribosomes with 17.5Å resolution. The relative orientations of ribosomes in polyribosomes and the mRNA pathways in each individual polyribosome were determined.

Conclusion: Cryo-ET in combination with sub-tomographic averaging is the most promising technique in cryo-electron microscopy. This approach in comparison of single particle analysis imposes additional requirements both at the stages of data acquisition and data processing. On the example of this work we demonstrated all the stages of the Cryo-ET pipeline. Taking into account the experimental equipment used, bottlenecks and possible solutions were shown.

Key Words: cryo-EM • cryo-ET • polyribosomes

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