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Single particle Cryo-EM of macromolecular complexes at near-atomic resolution.

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The ribosome is a complex cellular machine which consist of ribonucleic acids and proteins. It is the only macromolecular complex capable to decode the information from mRNA to the amino acid sequence of the protein in all kingdoms of life. The precise mechanism of action is still not clear. Our aim is to elucidate the molecular mechanism of partial reactions of the elongation cycle. In order to complete this task we have used high-end microscope Titan Krios equipped with Cs-corrector and high sensitive direct electron detector. This combination of equipment allows us to determine the structure of 70S E. coli ribosome at average resolution of 3.2A (some part of the ribosomal complex was resolved at 2.8A or better) in pre-translocational functional state.
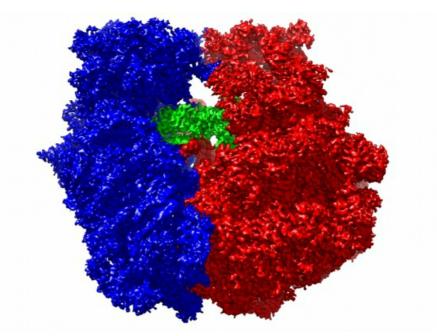
The sample preparation was as following: three microliters of 0.35 mg/ml 70S E. coli ribosome complex solution were applied onto carbon coated grids (Quantifoil R 2/2). In order to make grids hydrophilic and charge the surface negatively we pre-treat it by glow discharge in plasma cleaning device in presents of oxygen.

Vitrification process was performed in Vitrobot Mark IV (FEI) under 100% humidity and 4C temperature. The blotting force was setted to 5 and blotting time to 4 seconds. Grids was stored and transferred in liquid nitrogen.

Grids were loaded in the autoloader of Titan Krios microscope, precooled to the liquid nitrogen temperature. A total of 3783 images were collected in automated data acquisition mode in Cs-corrected cryo-TEM Titan Krios (FEI) equipped with XFEG-electron source and direct electron detector Falcon II using 300kV accelerating voltage, 75000x magnification (pixel size of 0.859A) and -0.6 to -3 um defocuses. Data collection took 7 days to complete.

The recorded movies were motion corrected using MotionCor2. Individual particles were initially picked manually using EMAN2 boxer subroutine in order to create templates. These templates were used for automatic selection of all the particles from all the collected images by gautomatch program. Subsequently, the 505830 picked particles were extracted in Relion 2.0.2 using a 512 pix box size. After performing 2D classification on binned by 8 data (C8-data) (pixel size 6.872A), ice- and contamination-free 2D class averages were selected. Extracted from these 2D classes particles were used for next rounds of image processing like two rounds of 3D classification. And only after with best particles

Figure 1. Cryo-EM density map of the 70S ribosomal complex at near atomic resolution.

Keywords: Cryo-EM, 70S ribosome