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Nucleotide-bound conformations of the GroEL-GroES1 complex resolved by cryo-EM

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The GroEL-GroES complex is a bacterial protein folding machine working in an ATP-dependent manner. Despite being studied for decades, details of its functional cycle remain under debate. One of the discussed topics is whether the two rings of GroEL function simultaneously (implying the formation of a symmetric "football-shaped" complex) or alternately (through an asymmetric "bullet-shaped" form). Both football- and bullet-shaped complexes were resolved experimentally using X-ray crystallography or cryo-electron microscopy, and the corresponding atomic models are available in the Protein Data Bank. However, the deposited cryo-EM structures of the GroEL-GroES complex are of 7 Å or lower resolution and often with an imposed C7 symmetry, which limits their interpretation. In this work, we have obtained a cryo-EM structure of the bullet-shaped GroEL-GroES complex at 4.3 Å with no symmetry applied. A sample for cryo-EM was prepared as follows: GroEL (1 μM) was incubated with GroES (3 μM) in 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM KCl, 10 mM $MgCl_2$, 3 mM ATP for 20 min. Then, the sample was concentrated 10 times using Vivaspin 500 with 100,000 MWCO PES membrane centrifugal filters. 3 μl of the sample was applied to a glow discharged grid (Quatifoil R1.2/1.3) and vitrified in Vitrobot Mark IV at 4.5°C. Cryo-EM movie stacks were recorded on a Titan Krios electron microscope (Thermo-Fisher) equipped with the direct electron detector Falcon II. 6100 image stacks were collected and further processed in Warp and CryoSPARC. 95208 particles were selected for the final symmetry-free 3D reconstruction. The local resolution of the equatorial domains reached 3.5 Å, which allowed us to distinguish extra densities in the ATP-binding pockets of both rings. The resolution variations also indicate that, compared to the GroES-bound ring, the second ring was more flexible at the apical domains. The authors acknowledge funding from the Russian Science Foundation (grant № 19-74-20055).

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RNA polymerase collisions with dCas9

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CRISPR-Cas systems serve as powerful tools for genome and epigenome editing and gene-specific transcription regulation. Cas9 is a programmable nuclease that has found its use in numerous in vivo and in vitro applications. It was shown that the catalytically dead Cas9 (dCas9) from *Streptococcus pyogenes* can suppress transcription of the target genes *in vivo* both in bacteria and in eukaryotes, presumably by binding to the DNA template and blocking RNA polymerase progression. Interestingly, the effect of dCas9 strongly depends on its orientation relative to the direction of transcription, suggesting that it may be differently recognized by the moving RNA polymerase. We are investigating the molecular mechanisms underlying these observations. We obtained purified dCas9 protein and several sgRNAs that direct dCas9 to different positions and orientations on a DNA template designed for *in vitro* transcription experiments. We demonstrated that dCas9 specifically impedes transcription by RNA polymerase from *Escherichia coli* at the sites of binding. Surprisingly, this effect did not depend on the orientation of dCas9. We further showed that transcription factors GreB and Mfd may facilitate transcription through dCas9 bound to the template DNA strand. We use a reporter system to determine whether transcription factors may cause asymmetry in dCas9 action on transcription in *E. coli* cells. The results contribute to our understanding of how RNA polymerase interacts with roadblocks during mRNA synthesis. This work was supported by the Russian Science Foundation (20-74-10127). AA is supported by Skoltech Systems Biology Fellowship.

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Analysis of a putative nuclease associated with the Argonaute protein from *Rhodobacter sphaeroides*

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The Argonaute protein from the alpha-proteobacterium *Rhodobacter sphaeroides* (RsAgo) binds small guide RNAs to recognize complementary DNA targets. In bacterial cells, RsAgo is bound to both small RNAs and small DNAs, with a preference for foreign genetic elements. RsAgo also promotes degradation of plasmid DNA *in vivo*. However, RsAgo itself lacks the slicer activity, suggesting the involvement of additional nucleases. To shed light on the molecular mechanism of target processing, we study a putative nuclease which is located in the same operon with RsAgo in the *R. sphaeroides* genome. This protein has motifs characteristic for the PD-(D/E)XK superfamily of nucleases. Numerous attempts to express the wild-type nuclease gene in *Escherichia coli* were not successful due to a low level of expression and low solubility of the recombinant protein. A codon-optimized sequence of the nuclease gene was cloned and successfully overexpressed in *E. coli*. Different expression vectors were used to produce histidine-tagged nuclease and a fusion protein with a chitin-binding domain, which allowed to obtain highly pure nuclease preparations. Initial assays did not reveal nuclease activity in these proteins. However, it was shown that co-expression of nuclease with the RsAgo protein in *E. coli* significantly increases the yield of RsAgo and changes the spectrum of genes preferentially targeted by RsAgo. We plan to further determine the role of nuclease in DNA processing in bacterial cells and test its interactions with RsAgo *in vitro* and *in vivo*. This work was supported in part by the Russian Science Foundation (grant 19-14-00359).