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Cryo-EM structure of the bullet-shaped GroEL-GroES complex at 3.6 Å resolution

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The GroEL-GroES complex is a bacterial protein-folding system that works in an ATP-dependent manner. Even though the complex has been studied for decades, the details of it's functional cycle remain to be the subject of debate. One important question is whether the two GroEL rings function simultaneously, which would imply the formation of a symmetric GroEL-$GroES_2$ ("football-shaped") complex [1], or alternately, through the formation of an asymmetric GroEL-$GroES_1$ ("bullet-shaped") form [2]. The structures of both forms were experimentally obtained by X-ray crystallography and cryoelectron microscopy (cryo-EM), and the corresponding atomic models are available in the Protein Data Bank. However, the GroEL-GroES structures obtained using cryo-EM possess a moderate resolution (7.7 Å or lower), which limits their interpretation. In this work, we resolved a symmetry-free cryo-EM structure of the bullet-shaped GroEL-$GroES_1$ complex at 3.6 Å resolution.

To prepare the sample, 1 μM GroEL was incubated with 3 μM GroES in 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM KCl, 10 mM $MgCl_2$, 3 mM ATP for 20 minutes at 20°C. Then, 3 μL of the sample was applied to a glow-discharged electron microscopy grid (Quatifoil R1.2/1.3) and plunge-frozen in liquid ethane using an FEI Vitrobot Mark IV at 4.5°C. In total, 6784 movie stacks were recorded on an FEI Titan Krios electron microscope equipped with a Falcon II direct electron detector. Motion correction, CTF estimation and particle picking were performed in Warp [3], then the particles were exported to Relion [4] for 2D and 3D classification. The final reconstruction was built from over 90,000 particles in CryoSPARC [5] with no symmetry imposed, its resolution 3.62 Å, as estimated using the FSC = 0.143 criterion (Fig. 1).

The obtained structure is characterized by variations in local resolution. The equatorial domains were resolved at 3.5 Å, making it possible to distinguish additional densities in the ATP-binding pockets of both rings. This differs our structure from previously solved X-ray and cryo-EM structures, where nucleotides were present in both rings only in the case of the football-shaped forms. The resolution of the intermediate and the apical domains varied from 4 Å to 6 Å, the lowest local resolution of 7 Å was observed in the apical domains of the trans ring. This implies the increased mobility of the apical domains in the trans ring or the presence of several conformations.

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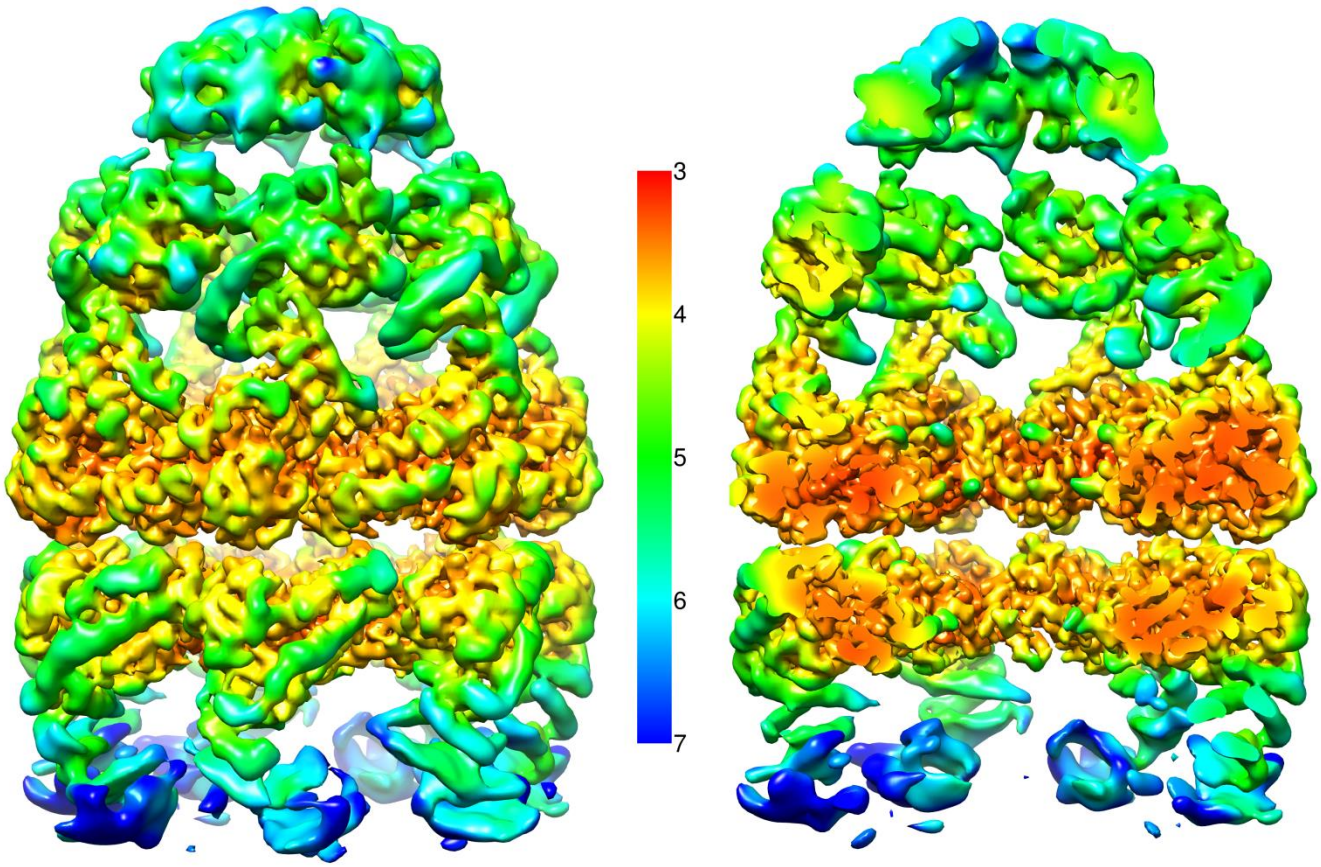

Figure 1. Cryo-EM structure of the bullet-shaped GroEL-GroES complex colored according to the local resolution. A surface view (left) and a slice through the center (right) are shown.

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