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Complex of Chaperonin GroEL–α-synuclein Resolved by Cryo-EM

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The accumulation of aggregated proteins causes neurodegenerative disorders, including Parkinson's, Alzheimer's, and prion diseases [1]. One cellular system designed to promote protein folding and prevent aggregation is the chaperone system, the modulation of its activity could prove to be a new approach for treatment of such diseases [2]. However, the interaction of the amyloidogenic protein with a chaperone can block the chaperone's ability of restoring the functional state of a substrate, as shown for the ovine prion protein PrP and the GroEL chaperonin [3]. Moreover, chaperonins of gut microbiota may be involved in pathological protein transformation in the gastrointestinal tract and the subsequent prion-like spread of infectious protein forms. For instance, fibrillar forms of α-synuclein can be transported from the gut to the brain, leading to the development of Parkinson's disease [4]. Thus, structural and functional studies of interactions between amyloidogenic proteins and chaperones can assist in exploring the onset of neurodegenerative disorders. Previously, using cryo-EM, we obtained the structure of the GroEL-PrP complex and studied it using the molecular dynamics simulations [5]. Here, we present the cryo-EM structure of a complex between GroEL and α-synuclein.

Recombinant human wild-type α-synuclein was produced in *E. coli* BL21(DE3) and GroEL was produced in *E. coli* W3110. In order to obtain the complex, 2 μM GroEL were co-incubated with 20 μM α-synuclein in TriS-HCl pH 7,5 on an Eppendorf Thermomixer comfort shaker for 15 minutes with constant stirring at 550 rpm at 23°C.

Automated experimental data acquisition was carried out using a Titan Krios 60-300 transmission cryoelectron microscope and EPU (FEI) software. The Warp software package was implemented for motion correction, estimation of Contrast Transfer Function (CTF) parameters, and particle selection. 2D classification and calculation of 3D reconstruction with no symmetry imposed were carried out using CryoSPARC. The resolution of the resulting structure was 3.63 Å according to the FSC=0.143 criterion.

For further analysis, the GroEL atomic model (PDB: 1SS8) was fitted into the 3D reconstruction (Fig. 1). An additional density corresponding to α-synuclein was observed in the region of the apical domains of the resulting structure. This density was identified only in one of the GroEL rings and was distributed between the apical domains of several neighboring subunits. Since α-synuclein is a disordered protein, the details of the secondary structure were indistinguishable. The area and nature of substrate binding in the chaperonin cavity resembles the result obtained for GroEL in complex with PrP [4]. Our results imply the common mechanisms behind the way amyloidogenic proteins interfere with chaperone functioning.

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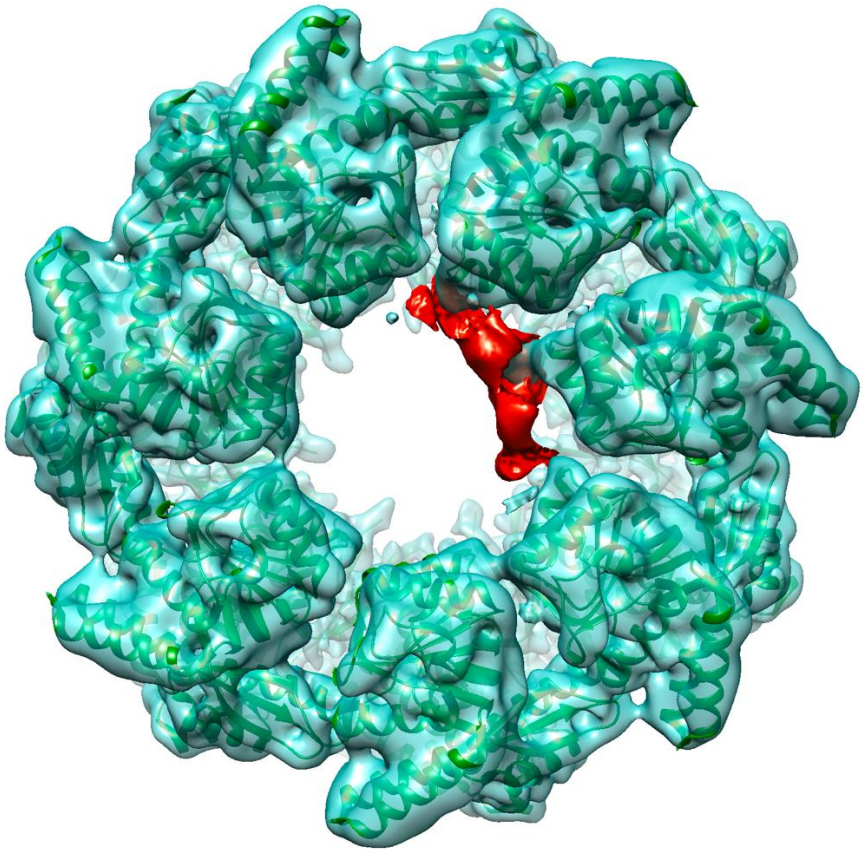

Figure 1. Cryo-EM structure of GroEL with α-synuclein. The atomic model for GroEL is shown in green, the region of the map corresponding to α-synuclein is shown in red.

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