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Conformational Dynamics of the CCT Protein Folding Machine

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The CCT ATPase complex (also known as TRiC) is a ~1 MDa protein folding machine that uses energy of ATP binding and hydrolysis to facilitate substrate folding. CCT plays an indispensable role in maintaining cellular proteostasis by assisting in the folding of many proteins with complex tertiary structures and unfavorable folding trajectories [1-3]. The complex consists of two sets of eight homologous, but distinct, subunits that assemble into a specific order to form a double-ring barrel shaped structure [4, 5] (Figure 1A). Each subunit consists of 3 domains: the apical domain, the intermediate domain, and the equatorial domain. The equatorial domain contains the ATP binding site and is involved in inter- and intra-ring subunit contacts [6] (Figure 1B). As each subunit binds and hydrolyzes ATP, it induces closure of the CCT barrel [7]. Yet, the mechanism of how conformational changes in CCT aids protein folding remains obscure.

Among the many CCT substrates are β-propeller proteins, including β subunits of G protein signaling complexes [8, 9]. G protein signaling complexes are the largest class of signaling molecules in eukaryotic cells and respond to a wide array of signals, including hormones, neurotransmitters, and photons of light [10]. The correct folding of G protein is essential to its function. However, little is known of how CCT assists in folding of Gβ5. It has been shown that expression of the CCT co-chaperone phosducin-like protein 1 (PhLP1) increases the binding between Gβ5 and CCT [11, 12], which suggests that PhLP1 works with CCT in the folding of Gβ5. The premise of this work is to understand how CCT and PhLP1 help the folding and release of Gβ5.

To investigate the folding process of Gβ5, we will determine the structures of CCT-PhLP1-Gβ5 in various conformations. We have purified CCT-PhLP1-Gβ5 complexes and prepared cryo-EM specimens in the presence of different nucleotide states. Imaging processing and 3D classification revealed a mixture of opened, semi-closed and closed CCT particles from a single dataset (Figure 2). Further processing using 3D variability analysis revealed several intermediate conformations ranging from 3.8 to 4.5Å. The conformations provide a more complete picture of CCT opening and closing. Ongoing efforts are underway to determine the continuous changes of substrate within CCT. The structures of CCT-PhLP1-Gb5 will provide insights into the folding mechanism of Gb5 and how CCT assists in the folding of β-propeller proteins.

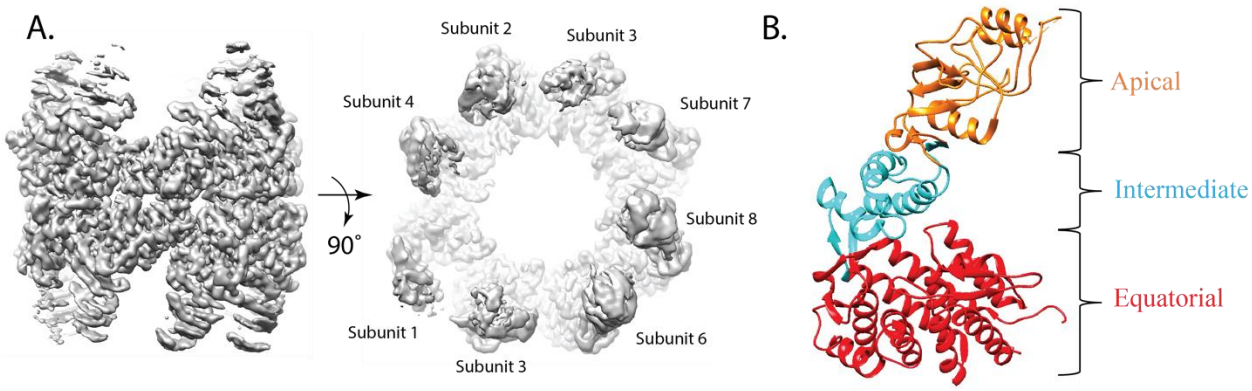

Figure 1. (A): CCT subunit arrangement. (B): CCT subunit domain organization.

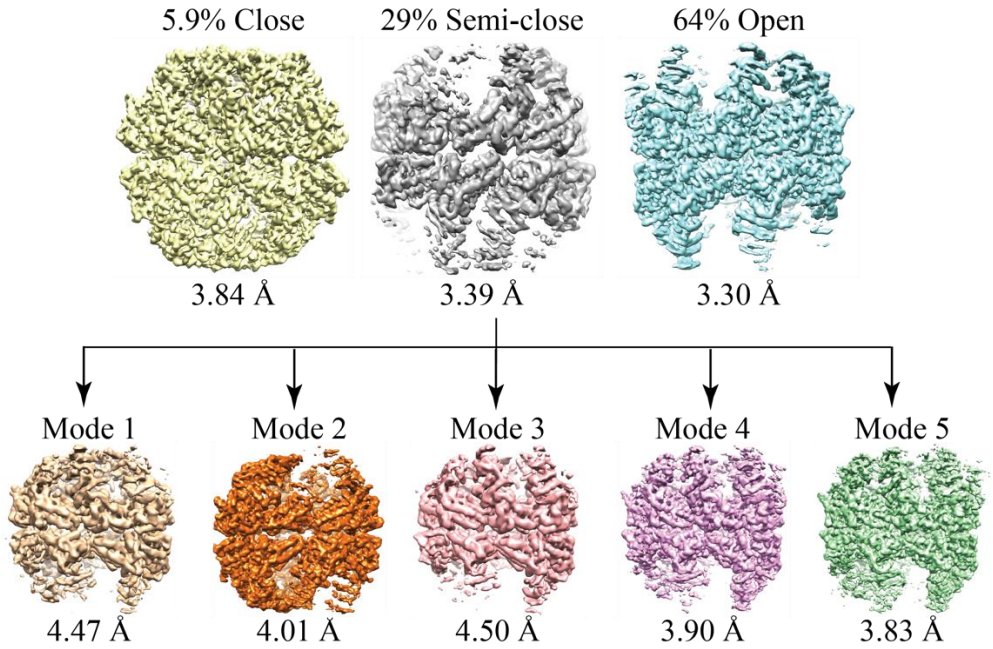

Figure 2. Main reconstructions of CCT- PhLP1- Gβ5 (yellow represents closed conformation, grey represents semi-closed conformation, cyan represents open conformation) and intermediate conformations results from 3D variability analysis of semi-closed conformation (gold represents mode1, orange represents mode 2, pink represents mode 3, purple represents mode 4, green represents mode 5).

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