

# Chaperonin GroEL-GroES action captured by high-speed AFM

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The correct folding of many proteins in prokaryotes and eukaryotes requires the action of large protein structures known as chaperonins. GroEL, the chaperonin of *Escherichia coli*, is composed of 14 identical 57kDa subunits forming two heptameric rings, stacked back to back, each with a large central cavity. The binding of ATP and the co-chaperonin GroES, composed of seven 10kDa identical subunits, to GroEL double ring is required for productive folding of misfolded proteins. The asymmetry in the binding-release reaction between GroEL and GroES has been resolved through biochemical studies, including single ATP turnover experiments and the analysis of single ring mutants. Based of these observations, a model for a GroEL-GroES reaction cycle has been proposed. The GroEL has a negative cooperativity between the two rings and the GroES is assumed to stably bind only to either GroEL ring during ATP hydrolysis. However, this alternate GroES binding has not directly been evidenced by single molecule experiments.

Here, we describe the direct elucidation of this negatively cooperative binding action of the chaperonin system using a high-speed atomic force microscope (high-speed AFM). We first prepared a sample system in which GroEL lay down on a substratum. Because GroEL has a nature to attach onto bare mica surface in an end-up orientation, which keeps GroES from accessing to the both ends of GroEL, an appropriate substrate was needed. For this purpose, we performed two-dimensional crystallization of streptavidin on a supported phospholipid bilayer. Over a wide area the obtained streptavidin crystal was flat enough for AFM observation. To anchor the GroEL molecules on a substratum at their sidewalls, a GroEL mutant and its modification with biotin is needed; Asp at the equatorial domain was replaced with Cys and biotin molecule was attached to this residue. With these preparations it became possible to observe GroEL from its side by high-speed AFM. GroEL alone looked like a barrel, while GroEL associated with one GroES looked like a bullet. In the presence of GroES and ATP, dynamic changes in the GroEL appearance were successfully captured on video. The kinetics of dissociation of GroES from GroEL depicted from high-speed AFM observations showed two rate constants, contrary to the conventional model that assumes only one rate constant. Furthermore, a football shaped complex, in which the GroEL is bound by two GroES at the both ends, was confirmed to be formed during the chaperonin reaction cycles. We will report the details of these studies.