A chaperonin protein, GroEL, has a more complex mechanism than was thought before

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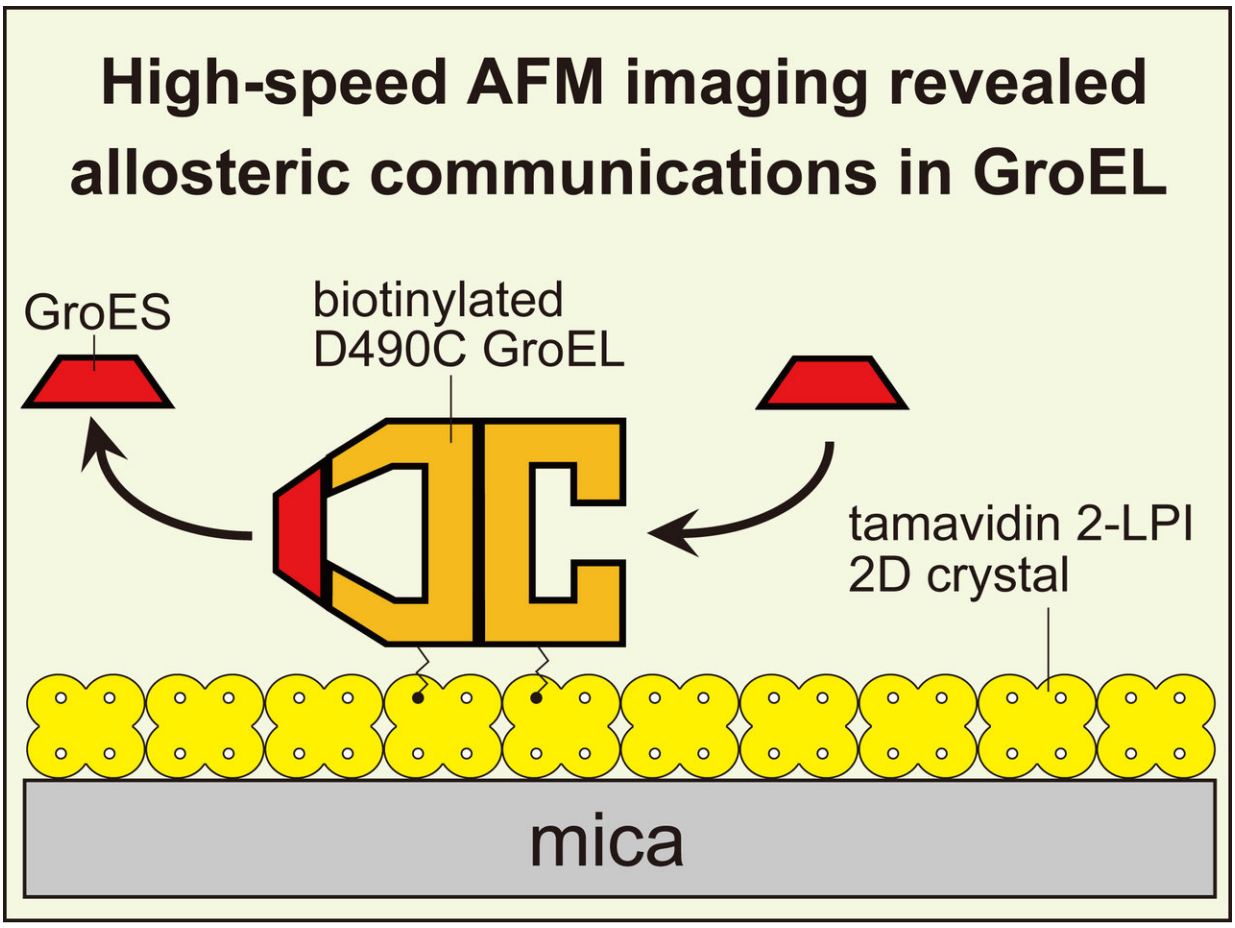


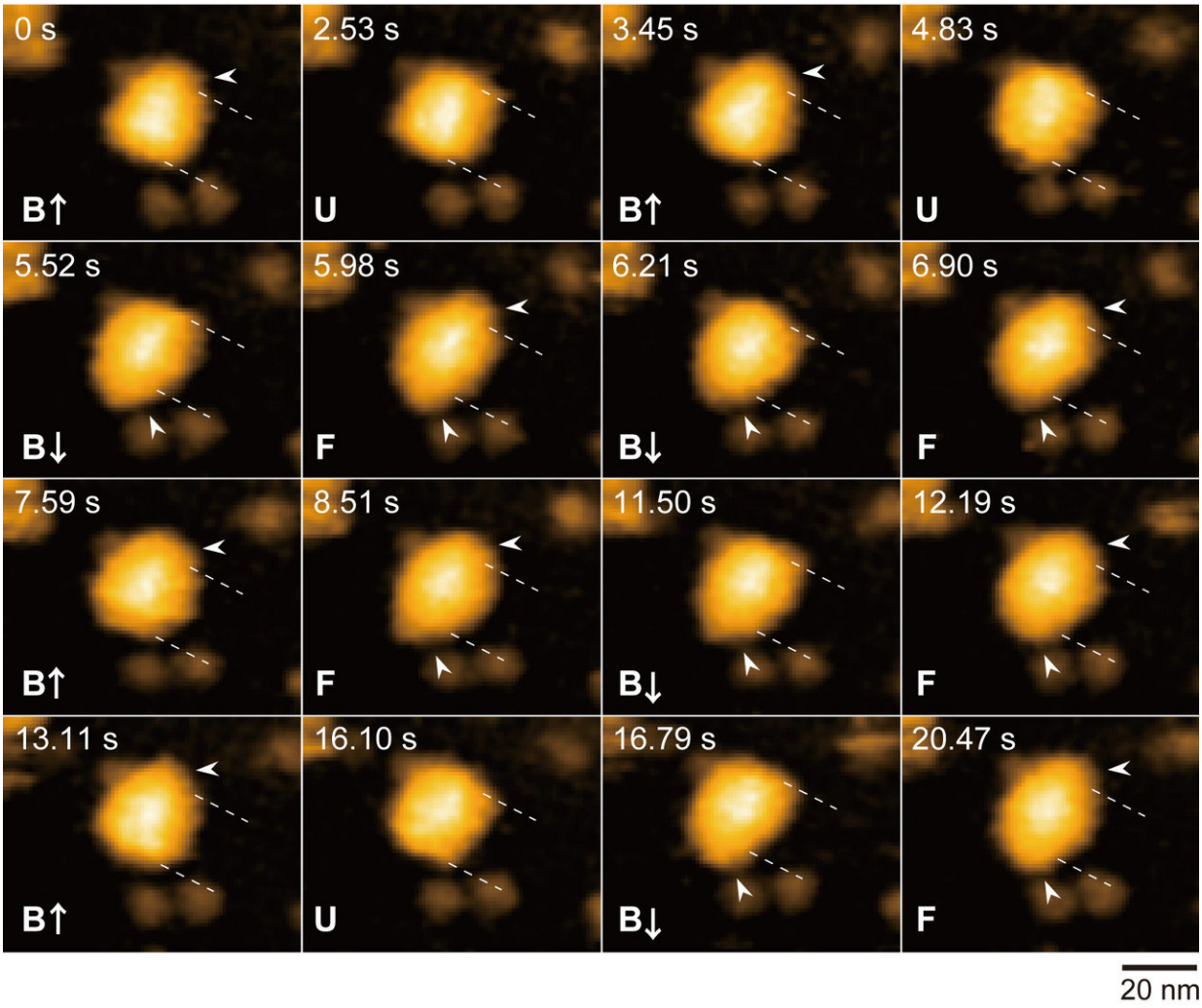
HS-AFM imaging revealed allosteric inter-ring communications in GroEL governing its chaperonin reaction. To visualize dynamic GroEL-GroES interactions with high-speed AFM, GroEL biotinylated at its equatorial domains was tethered to the two-dimensional crystal of tamavidine 2-LPI directly formed on mica surface. Credit: Kanazawa University

Proteins must fold in a specific way to function. This is often assisted by molecular chaperones—small proteins whose job is to help others fold to the right shape. Now, Japanese researchers have discovered that for one molecular chaperone at least, there's more to the process than was suspected.

In a paper in *Philosophical Transactions of the Royal Society*, the Kanazawa-led group focused on GroEL, which is vital to protein folding in bacteria. The rough outline is understood: GroEL captures an unfolded target protein (the substrate) within a cavity, where it can fold correctly without aggregating. However, the mechanistic details are hard to unravel with traditional ensemble methods. In the new study, high-speed atomic force microscopy (HS-AFM) was used to visualize events more directly.

GroEL is a cylinder-shaped molecule, made of two rings stacked back to back. A key partner in its function is GroES, a ring-shaped "co-chaperonin" that binds to each end of GroEL like a domed lid. Only when GroEL is capped by GroES can it trap the substrate protein. Then, when folding is complete, GroES dissociates from GroEL, and the folded substrate is released.

Where it gets hazy is how the two rings at either end of GroEL cooperate. The rings are identical, and both can be capped by GroES. When only one end is capped, the resulting complex is termed a "bullet", by virtue of its pointed appearance. Meanwhile, the form with both ends capped is dubbed a "football", as its symmetrical oval shape resembles a gridiron ball.



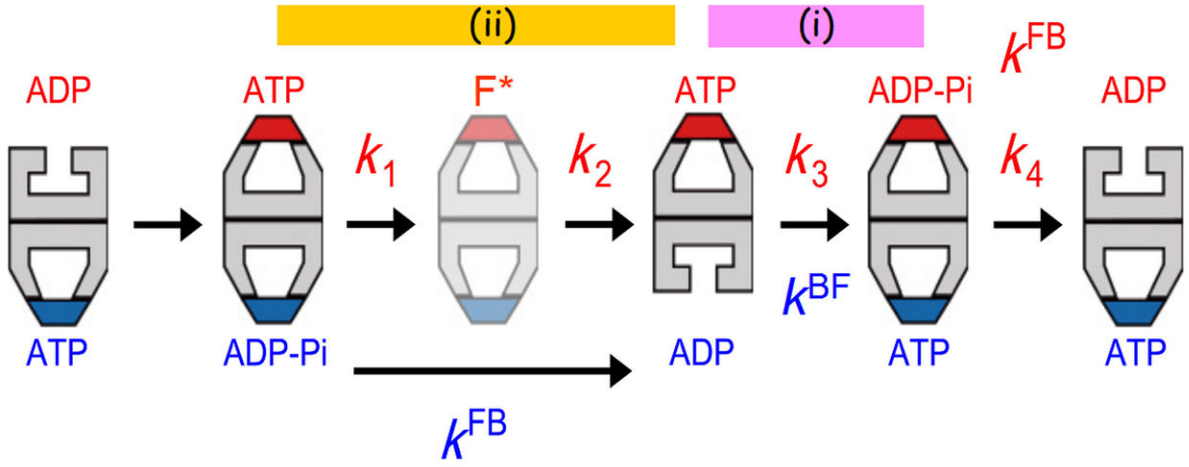
GroES association and dissociation events captured by HS-AFM imaging. Although the reaction mainly proceeds in an alternate fashion as B? ? F ? B? ? F (B and F represent bullet and football complexes, respectively, and the vertical arrows indicate the polarity of bullet complexes), it occasionally occurs in different ways. Credit: Kanazawa University

"In one conventional model, the cycle of capping, protein folding, and uncapping alternates between each ring," says study co-author Daisuke Noshiro. "Capping at one ring of GroEL (which has cis stereochemistry) prevents simultaneous capping at the other (trans) end. Such

intramolecular communication is known as allostery." In this view, the single-capped bullet is the active form of GroEL, and the football is merely a short-lived intermediate between cycles.

Other findings, though, have hinted at greater complexity— which has been highlighted by this new study. Depending on the substrate type, GroEL appeared as a football, rather than a bullet, up to 67% of the time, implying a breakdown of negative allosteric regulation. This was most common when the substrate was an unfoldable protein or there was no substrate at all, but even with foldable substrates, football complexes abounded.

More unexpectedly, the cycle occurred by two different pathways. In the predominant Type I, when the active ring of GroEL completes its task and the other end takes up the baton, the two rings also exchange cis and trans conformations. However, around 25% of the time (in Type II), the conformations are not exchanged, disrupting the circular, alternating rhythm of Type I. Nonetheless, protein folding still occurs. Footballs are prevalent in both cases.



Allosteric communications between two rings of GroEL. The lifetime

distribution of bound GroES was best fitted to a sequential four step reaction model with four rate constants, k1, k2, k3 and k4. The value of k3 agreed with that of the rate of bullet-to-football transition (kBF) in the opposite ring. Moreover, the value of 1/k1 + 1/k2 agreed with that of 1/kFB (kFB, the rate of football-to-bullet transition in the opposite ring). The former agreement indicates that ATP hydrolysis into ADP-Pi on one ring acts as a time keeper for ADP release from the opposite trans ring, ensuring the release of substrate protein from the trans-ring before it is capped with GroES. The latter agreement indicates that an event occurring in the second step after ATP binding triggers Pi release from the opposite ring. Credit: Kanazawa University

"The football structure is so abundant, it must play a more active role than we thought," says corresponding author Toshio Ando. "This complex mechanism is important, because chaperonins are a natural class of molecular machines. The subtleties of GroEL may help us to understand the role of allostery in molecular machines more generally."

More information: Daisuke Noshiro et al, Substrate protein dependence of GroEL–GroES interaction cycle revealed by high-speed atomic force microscopy imaging, *Philosophical Transactions of the Royal Society B: Biological Sciences* (2018). DOI: 10.1098/rstb.2017.0180

Provided by Kanazawa University