【Grant-in-Aid for Specially Promoted Research】
Biological Sciences

Title of Project : Beyond single-molecule physiology: Letting molecular machines work by soft force

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Research Area : Biological Sciences, Biological Science, Biophysics
Keywords : Single-molecule measurements and manipulation, Structure, dynamics and functions of proteins and nucleic acids, Nanomachines

【Purpose and Background of the Research】

We aim at elucidating mechanisms of 'molecular machines' such as ATP synthase that rotates inside our body to produce ATP and ion channels that generate electric signals in neuronal networks. The best method has been 'single-molecule physiology' where one closely watches in real time how individual molecules work and, if necessary, applies a force to see how they respond. Observation alone, however, is often inconclusive, and forces have so far been applied in a negative fashion, breaking, stopping, or impeding a machine.

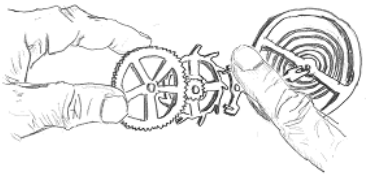

Fig. 1. A search by fingers.

If one is to find the mechanism of a complex machine, one would let it work (move) by fingers (Fig. 1). The touch must be soft. The machine will move smoothly if a force is applied on a right spot in the right direction, else the fingers will tell how one has erred. We will do the same for a molecular machine, to grasp its mechanism at once. We will deprive the machine of its natural energy source such as membrane potential or ATP, and ask if an external force can substitute. Or we will impair part of the machine and try to restore its function by an assisting force.

【Research Methods】

The single-molecule physiology we have been practicing so far relies on a probe that is 'huge' compared to the nanometer-sized molecular machines, such as a micron-sized plastic bead or an actin filament. We aimed at seeing-is-understanding type experiments under an optical microscope, where the huge probe reveals the molecular behavior directly without requiring complicated analysis.

In this proposal, a huge probe will serve as a handle through which a soft force is applied, by magnetic or optical tweezers, or flow. For example, we attach a magnetic bead and rotate ATP synthase (Fig. 2) to let it synthesize ATP without proton gradient or pump protons without ATP.

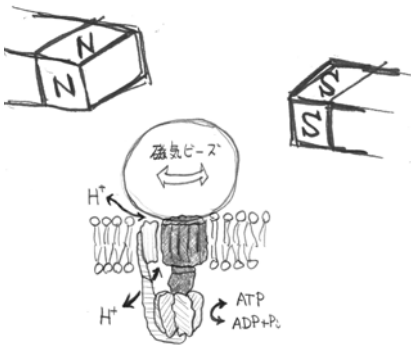

Fig. 2. Magnetic assistance.

【Expected Research Achievements and Scientific Significance】

The eventual goal is to open up a new field of science, active single-molecule physiology. Letting a defective molecular machine work by an external force is a formidable task, but watching a single molecule was nothing but a dream until recently. Creating novel functions by assisting forces is another goal we aim at.

【Publications Relevant to the Project】

S. Furuike *et al.* "Axle-less F_1-ATPase rotates in the correct direction" ***Science***, 319, 955-958 (2008).
K. Adachi *et al.* "Coupling of rotation and catalysis in F_1-ATPase revealed by single-molecule imaging and manipulation" ***Cell***, 130 309-321 (2007).
K. Shiroguchi & K. Kinosita, Jr. "Myosin V walks by lever action and Brownian motion" ***Science***, 316 1208-1212 (2007).

【Term of Project】 FY2009-2013

【Budget Allocation】 474, 900 Thousand Yen

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