Templated Self-Assembly of Biomolecules with High Spatial Precision

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Bio-molecular machines are nanoscale molecular complexes that perform important biological functions in ensemble, despite their seemingly unrelated behavior in the cell. Understanding how individual machines cooperate productively has become increasingly important for understanding mechanisms that enable the macroscopic functions. In this paper, I briefly review the recent advances in experimental techniques for studying these biomolecular interactions.

1 Introduction

It is well known that living organisms communicate and process information with surprisingly low energy consumption; however, not only that, they also demonstrate advanced information processing including self-organization, self-duplication and adaptability that are not achieved by current human technology so far. Surprisingly, they do all of this in the course of their daily lives as they duplicate themselves from one generation to another. Understanding and application of the underlying mechanisms would allow us to achieve a major breakthrough in human prosperity. Nonetheless, in order to understand these living systems that are consisted of tiny biological molecules, we should start by addressing how these systems are designed from the molecules.

X-ray crystallography and the single-molecule imaging technique which became popular around 1990 clarified an aspect of biomolecules as biomolecular machines, and it has so far been revealed that biomolecules such as proteins and RNA behave just like microscopic machines that perform a variety of functions. It is known that these "biomolecular machines" are made of string-like polymers, which become folded autonomously to form structures with set shapes that catalyze chemical reactions with astounding efficiency, or transport microscopic substances along rails, and play a variety of other roles.

In this way, we are gradually building up an understanding of the functional mechanisms of individual biomolecular machines, but in spite of this, there is still a huge gap in our understanding between the flexibility and homeostasis demonstrated by biological systems and our understanding of biomolecular machines at the single molecular level. In other words, although it is needless to say that organisms demonstrate the characteristics of superb autonomous systems, we have no understanding whatsoever of the true nature of the mechanisms behind how these components are organized into systems. One hypothesis about these systems is that the mechanism for self-organization is incorporated into each of the components, with the separate components influencing the whole, and vice versa in a feedback system, which eventually settles on a particular structure (including not only physical, but also network structures)[1]. For example, it is thought that the appropriate designing of these feedback loops gives organisms the flexibility to adapt necessary periodic functions in vivo to external cycles, while simultaneously having robust mechanisms that cleverly avoid being influenced by pulse-like external perturbations. Systems like these are known to have complexities that do not allow deterministic predictions of results when the varieties of components in the system increase. Therefore, it is often impossible to predict the characteristics of components within a system even though the mechanisms of individual components may have been clarified in detail. Gaining an understanding of such complex systems requires examination of how the microproperties of biomolecular machines interact with the macroproperties of the system as a whole. What is needed to achieve this is an experimental system that enables rapid experimental cycles in which the entire system can be tested upon modifying individual components, just as in wind tunnel testing frequently conducted in designing airplanes. In this type of experimental system, it is important to control parameters as precisely as possible regarding how and which type of

component to integrate.

2 Technology for accurately positioning biomolecular machines

A problem with creating this type of experimental system is the small size of biomolecular machines. Small biomolecular machines may only be a few nanometers across, and even bigger ones are only around several hundred nanometers. Positioning these on a substrate with nanometer precision is extremely difficult even with the use of robots and micromanipulators. We decided to use DNA nanostructures, which are being developed in recent years, as scaffolds to try and prompt self-organization by simply mixing them with biomolecular machines. Among DNA structures, a structure known as DNA origami, developed in 2006 by P. Rothemund of CalTech in the U.S., involves a single strand of DNA extracted from a natural virus, which is folded into a predetermined shape using approximately 200 varieties of short DNA fragments to create a set structure of around 100 nanometers squared. This method is characterized by extreme freedom of design compared to traditional methods of building DNA nanostructures[2]. Figure 1 shows DNA origami designed with a tube shape with 15 kinesin molecular motors, a type of bio-molecular machine, positioned on it at roughly 30 nanometer intervals. The key to positioning molecular motors on the DNA scaffold is a mechanism known as the enzyme tag system attached to both the DNA and molecular motors. This system is designed to allow only compatible parts to bond, much like the way a key fits into a key hole. Prior attachment of these to the DNA and molecular motors enables the desired molecular motors to attach themselves to the desired positions simply by mixing the two (Fig. 1). It is possible to chemically synthesize DNA, and it is also possible to attach a specific functional group to a part of the DNA sequence. This means that it is easy to attach the "keyhole" substance of an enzyme tag system to a specific location on the DNA origami. Furthermore, prior manipulation of the DNA to make the "key" or enzyme tag of the enzyme tag system bond with molecular motors enables the mass production of molecular motor-enzyme tag components.

3 Technology to assess molecular motion

The molecule known as a molecular motor used in our experiment moves unidirectionally in the cell, on protein filaments that serves as rails, using chemical energy. It is the mechanism on which muscular movement and

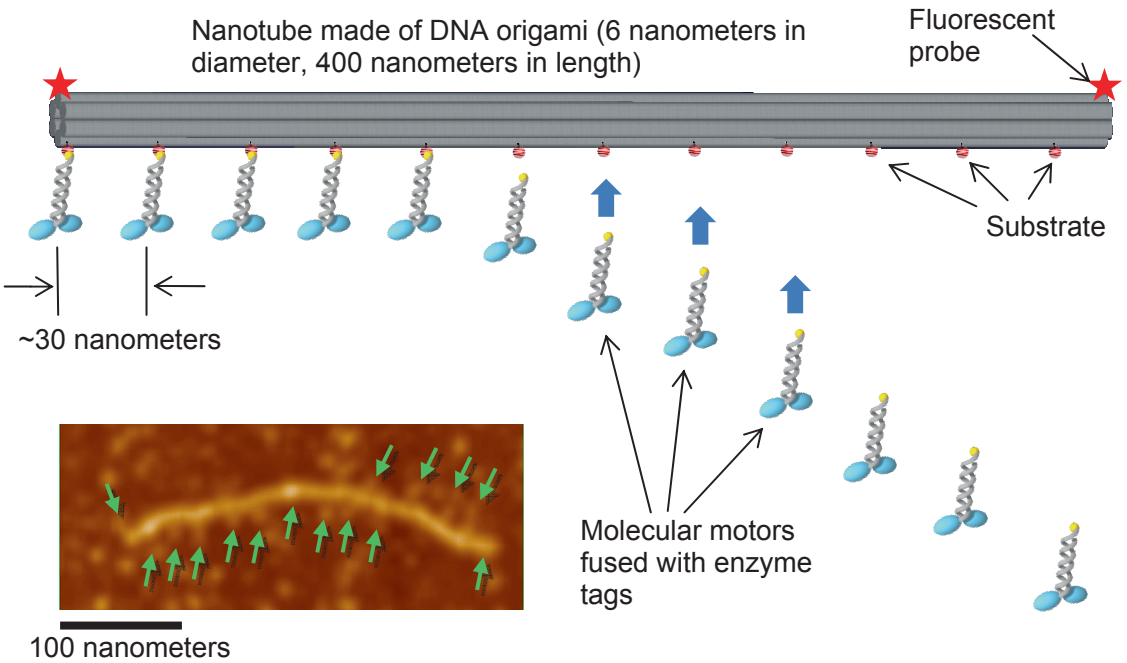

Fig. 1 Self-organization in building a molecular motor complex
Above: Artificial substrate (ligand) recognized by enzyme tags is attached to a fragment of a single strand of DNA attached to a DNA origami structure, which serves as the template. This is mixed with molecular motors with enzyme tags attached to their ends to build molecular motor complexes through self-organization.
Below: An AFM image of a molecular motor complex. Molecular motors are attached at positions indicated by the arrows.

substance transportation is based, and high expectations are held for it as a new source of power and for application in communication devices that make use of characteristics unique to organisms such as low energy consumption and high noise tolerance. Despite the fact that molecular motors are usually known to operate in groups consisting of multiple molecules, little is known about the characteristics of such molecular complexes. The reason for this is because it is extremely difficult to control the number and positioning of extremely small molecules. In order to bridge the gap between our understanding of a single molecule, and that of multiple molecules, we developed new technology to position molecules using DNA as a scaffold. We then measured the output of systems (transportation speed, distance and force generated) with one, two, three and four molecular motors using an advanced imaging technique[3]. The techniques used were the total internal reflection fluorescence microscopy for observing the fluorescence dye attached to molecules, and the use of the optical trapping apparatus, which can measure force at piconewton levels. Total internal reflection fluorescence microscopy utilizes total internal reflection at the interface between water and glass due to the difference in their refractive indices. When this happens the light permeates the water (to a depth of several hundred nanometers) to excite the fluorescence dyes in the vicinity of the glass resulting in an extremely low-level background and allowing the detection of fluorescence from a single molecule[4]. Fitting a 2-D Gaussian function to fluorescent spots projected on an ultrasensitive CCD camera achieves positioning precision with about 1 nanometer, far surpassing the accuracy imaginable from the resolution of normal fluorescence microscopes[5]. However the time resolution is around several dozen milliseconds, making it unsuitable for assessing phenomena such as the conformational changes of molecular motors that occur on fast time scales. On the other hand, the optical trapping apparatus has an objective lens with a large numerical aperture, which focuses laser light to trap microscopic objects on a micrometer scale. It is an optical microscope that enables free manipulation of sample objects by moving the position of the laser light[6]. Trapped microbeads can act like simple springs centered on the positions where they are trapped, enabling them to be used like probes for measuring force (Fig. 2). Extremely high spatial resolution is achieved as the result of a microbead image being projected onto a quadrant photodiode to examine the differential amplifier output (sometimes below

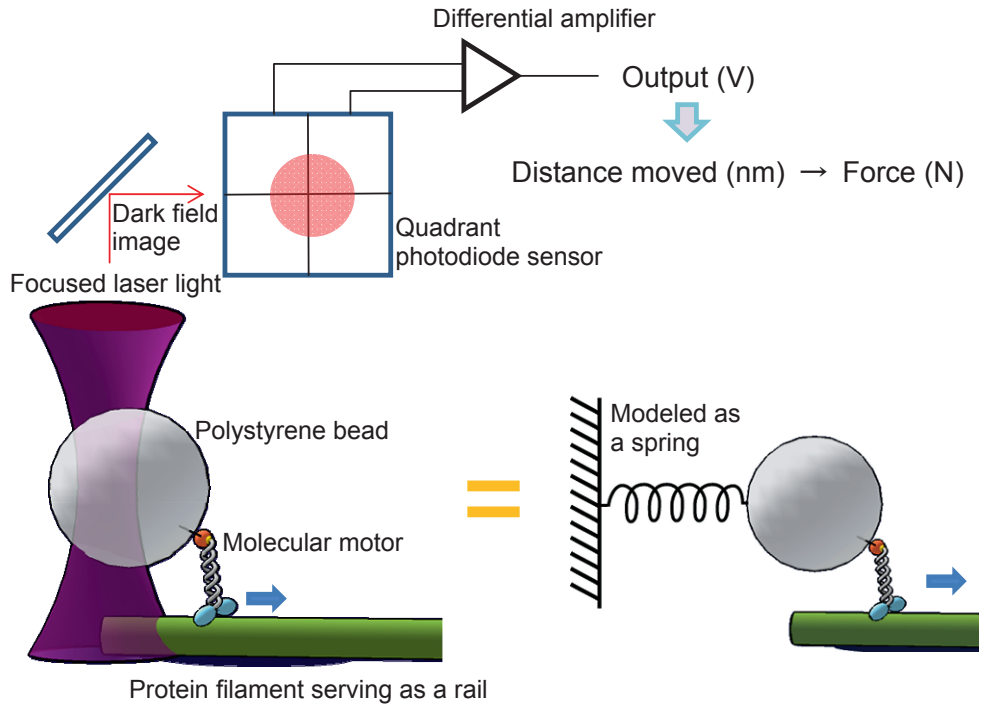

Fig. 2 Evaluating the performance of a molecular motor using the optical trapping apparatus
A polystyrene bead is trapped by focused infrared laser light to measure the amount of force generated by a molecular motor. The signal detected by a quadrant photodiode sensor is passed through a differential amplifier to convert it to information on how far the bead has moved. The optical trapping apparatus is similar to a spring, so it can be used to ascertain the amount of force generated by the molecular motor according to Hooke's law.

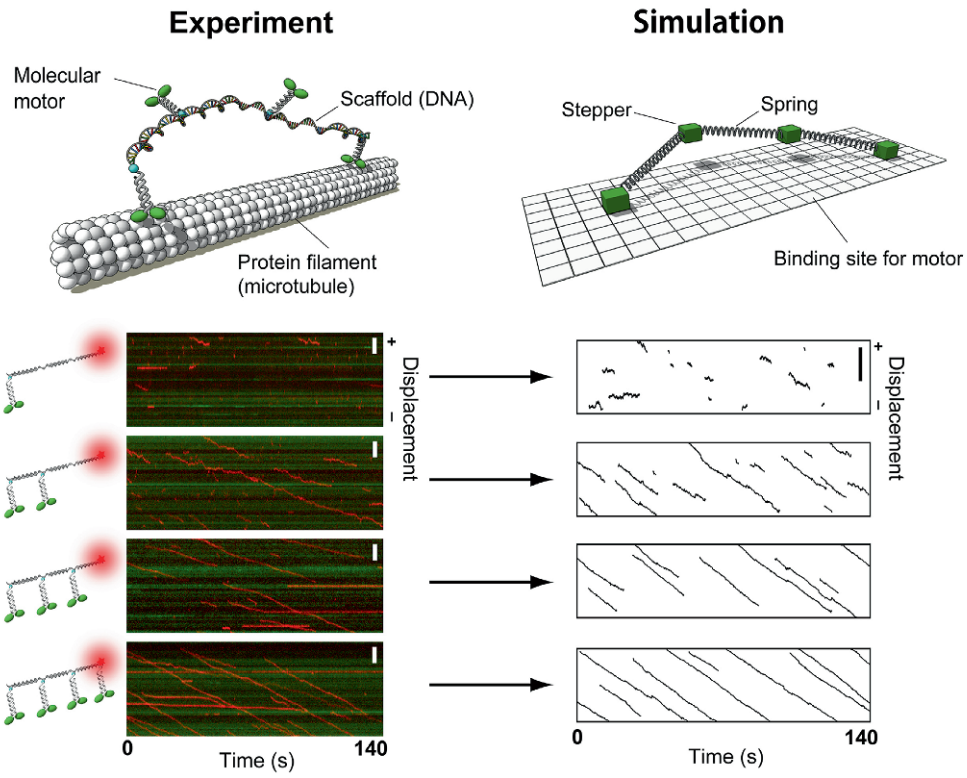

Fig. 3 The movement of molecular motor systems on DNA scaffolds and model simulations
Left: Schematic diagrams of molecular systems and their trajectory of motion along microtubules. Continuous movement along the microtubule was observed the moment the number of molecular motors (Ncd molecules) making up the system increased from one to two. The red lines indicate their trajectory of motion.
Right: A diagram of the model and the trajectory of motion reproduced through simulations. The major change in continuous movement as the number of molecular motors was increased from one to two was reproduced.
Bars = 3 μm.

a nanometer depending on conditions). In addition, the time resolution is in the order of magnitude of microseconds, allowing it to follow the dynamic changes in condition of molecular motors.

4 The characteristics of molecular motor complexes

As the result of using these techniques to assess the motion of molecular motor complexes, we discovered that types of molecular motors that cannot move efficiently on their own are able to function extremely efficiently as two coupled molecular complexes (Fig. 3). Furthermore, the transportation performance of multiple molecular motors is inversely proportional to the exponential function of the distance between the molecules, revealing that molecular coupling has a major influence on the performance of molecular motor complexes. Some unexpected results were that types of molecular motors that do not perform very well on their own become relatively powerful in a group. Their performance improves uniformly, whereas with types of molecular motors that perform well on their own, they do not perform much better in groups than they do on their own because the molecules interfere with each other. This indicates that organisms use molecular motors designed for working in groups, and those designed for working on their own for different purposes depending on where they are used. Moreover, it has come to light that in another type of molecular motor, an autoregulatory mechanism is triggered only when multiple molecules come together to form complexes. With this type of molecular motor, it assumes a form that does not allow efficient movement most of the time as a separate solitary molecule, but when multiple molecules gather to form a complex, the molecular motors activate each other to function more efficiently as a complex. The key to this system is the mechanical interactions between molecules, which activate each molecule, and the activated molecules further activate other molecules to form a positive feedback loop. This is thought to be what creates stable

transportation functions. This indicates that each molecule is equipped with control mechanisms for regulating the complexes as a whole, enabling functions to be switched on by the mere gathering of molecules in the absence of an obvious central control system, and this is the kind of system cells make ingenious use of.

5 Prospects for the future

If it were to become possible for people to design a true bottom-up system incorporating a mechanism within components for controlling a complex as a whole, it would overcome the vulnerability of a top-down system which collapses the moment the central control system breaks down. It would allow the creation of autonomous artificial systems that respond to the environment with flexibility. However, the reality is that the design principles of such non-linear systems are not self-evident. What we must do is try to mimic organisms that already have astounding, high-performance bottom-up systems by trial manufacturing numerous artificial systems to gather data, and build fundamental theories through induction. Our forebears observed apples falling from trees and the periodic motion of the planets, then used the data to build theories of mechanics through induction. In the same way, we will implement experimental cycles of building and observing systems using biological components in our research, which we believe will lead to the establishment of fundamental theories required for creating new biomimicry devices.

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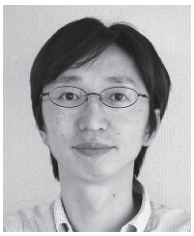

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