Development of a Molecular Robot Equipping a Shape Changing Function in Response to Signal Molecules

	SATO YUSUKE
	Tohoku University
	18149
URL	http://hdl.handle.net/10097/00125267

さ とう ゆうすけ
氏　　　　　名　佐 藤 佑 介
研究科，専攻の名称　東北大学大学院工学研究科（博士課程）バイオロボティクス専攻
学 位 論 文 題 目　Development of a Molecular Robot Equipping a Shape Changing
Function in Response to Signal Molecules
論 文 審 査 委 員　主査　東北大学教授　村田　智　　東北大学教授　西澤　松彦
東北大学教授　石川　拓司　東北大学准教授　野村　慎一郎

要約

Robots generally exhibit complex behavior though sensing, processing, and actuating function based on information which robot recognized. In other words, a robot can be defined as a system into which devices such as a sensor, processor, and an actuator are integrated into an independent body.

The rapid progress of molecular biology and nanotechnology has enabled us to understand the properties and functions of various biomolecules, including nucleic acids, proteins, lipids. Recent strides in these fields have allowed researchers to modify biomolecules and apply them for the construction of various molecular "devices," including sensors, processors, and actuators. For example, sequence-designed deoxyribonucleic acid (DNA) can be used as a material for the construction of arbitrarily shaped structures, or for computing or sensing. Motor proteins have been used for the development of actuators at the molecular scale. Moreover, cell-sized giant liposomes can be easily prepared and are widely used as drug carriers or artificial cell models. DNA nanostructures capable of functioning on liposomal membrane have been developed, and methods for the encapsulation of biomolecules, such as DNA and proteins, into giant liposomes are well established. Thus, if molecular devices are consistently integrated into a body of a giant liposome, the concept of a "molecular" robot could potentially be realized.

However, the integration of the molecular devices is challenging because each biomolecule functions optimally under a particular set of conditions, and their behaviors can be influenced by slight alterations to these conditions. Moreover, if this assemblage of molecular devices is to be termed as a "robot," then it should be able to decide its own behavior based on the inputted signals. Therefore, to realize the concept of a molecular robot, it would be necessary to integrate molecular devices into a compartment and facilitate function capable of determining behavior in response to external signals.

I herein describe an amoeba-type molecular robot equipped with a shape-changing function that responds to signal molecules. This molecular robot was developed by integrating molecular actuators and actuator controlling devices, termed a "molecular clutch", into a giant liposome. The actuator comprises kinesin and microtubule proteins. The molecular clutch was made of sequence-designed DNA molecules. When the clutch is an engaged state, it transmits the force generated by the actuator to the liposomal membrane; however, once the clutch is disengaged, the force (generated by the actuator) cannot be transmitted. The engagement/disengagement of the clutch can be controlled by inputting signal DNAs with particular base sequences, which

result in the initiation and termination of the shape change in response to signal DNA (Figure 1).
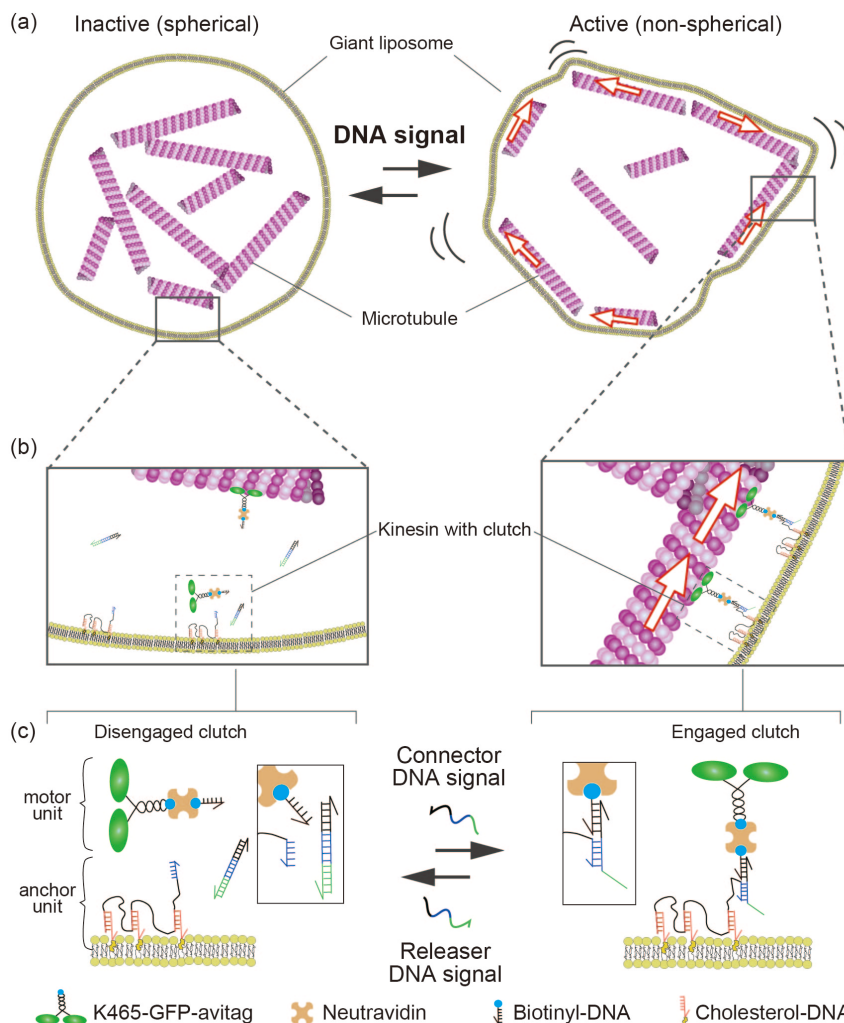

Figure 1. Schematics of our design for an amoeba-type molecular robot. (a) Overall schematics of the robot in its inactive (left) and active states (right). (b) Magnified schematics of the liposomal membrane when kinesins are detached from (left) or attached to (right) the membrane. (c) Schematics of the clutch mechanism indicating the disengaged clutch (left) and the engaged clutch (right).

In the process of equipping the amoeba-type molecular robot with a shape-changing function, I found that the lipid membrane composition was essential for facilitating shape change. This molecular robot exhibited a dynamic shape change, as a function of the molecular actuators only when the clutch was engaged. Furthermore, using photo-responsive DNA signals, initiation and termination of the shape change in response to the signal DNA were successfully demonstrated. The robot terminated the shape change after the input of the signal DNA via photo-irradiation. In addition, the reverse process—that is, initiation of the shape change by input of a signal was also demonstrated (Figure 2).
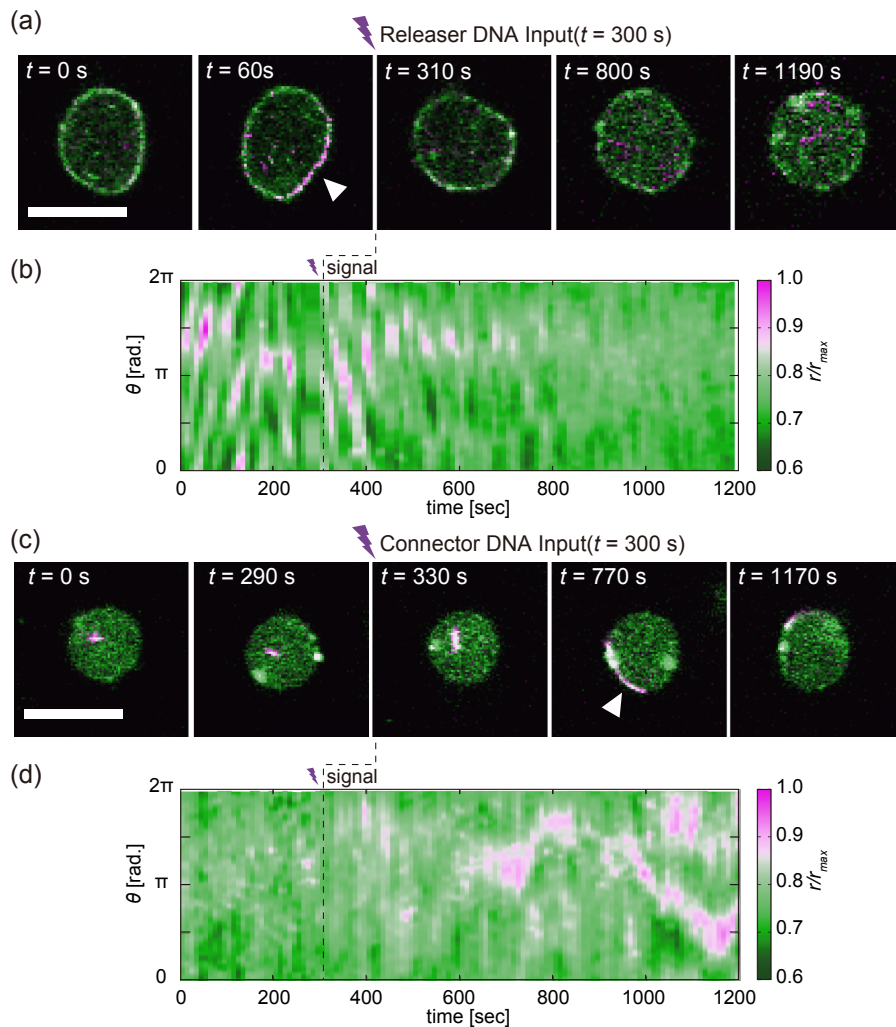
Figure 2. Switching the shape of an individual robot. (a) Robot image sequences transition from active to inactive: the releaser DNA signal was inputted at $t = 300$ s via UV irradiation; the white arrow head at $t = 60$ s indicates the microtubule attachment on the membrane. Green and magenta show kinesins and microtubules, respectively. Scale bar: 10 μm. (b) Color map of r/r_{max} of the inactive robot shown in (a) Here, r and r_{max} represent the distance from the center of mass to the membrane and the maximum value of r during observation, respectively. (c) Robot image sequences show the transition from inactive to active: the connector DNA signal was also inputted at $t = 300$ s via the irradiation, and the white arrow head at $t = 770$ s indicates the microtubule attachment on the membrane. Green and magenta show kinesins and microtubules, respectively. Scale bar: 10 μm. (d) Color map of r/r_{max} of the inactive robot shown in (c).

I also implemented a DNA amplification mechanism in giant liposomes to control the robot in the presence of few signals. The performance of the amplification mechanism in test tubes and in giant liposomes was also evaluated. My findings show that DNA comprising particular base sequences were amplified in the giant liposomes by premixing trigger DNA that initiated the amplification reaction. This amplification process yielded 500 times the amount DNA and the took approximately 40 min. It was revealed that the amplification characteristics were different between in a test tube and in a giant liposome; namely,

the amplification velocity in giant liposomes were lower than that in the test tube. In addition, this amplification within a liposome could be also initiated by external photo-stimulation.

For the development of the molecular device to directly input signal molecules from outside of the robots, I investigated the dynamic behavior of DNA origami nanostructures on lipid bilayer membrane. I found that the DNA origami on phase-separated lipid bilayer membrane prefer to localize on the solid-ordered (So) phase in the buffer condition used for the preparation of DNA origami. In addition, these nanostructures can form lattices via 2D self-assembly on the liquid-disordered (Ld) phase, but were adsorbed in a disorderly manner onto the So phases owing to the higher charge density. By changing the NaCl concentration in the buffer solution, the DNA origami detached from Ld phase and the disordered aggregation on So phase transformed into 2D lattices. These results suggest that by adjusting the NaCl concentration, the interaction strength between the DNA origami and lipid membranes can be tunable.

Thus, based on the experimental results described above, I designed and constructed a DNA origami device with the needle-like structure to input DNA signal by penetrating the lipid membrane. I successfully confirmed the folding of the DNA origami devices via agarose gel electrophoresis and direct visualization, using atomic force microscopy. However, the input of the DNA inside the liposome was not observed, because the needle did not penetrate lipid bilayer membranes. That may be due to an undesired interaction causing aggregation of the DNA origami devices. Therefore, further investigation of suitable experimental condition would be required in order to avoid this aggregation, which, in turn, should allow for the penetration of the needle and subsequent control of the amoeba-type molecular robot through externally generated molecular signals.

Overall, the results reported here provide a useful platform for the study of molecular robots and as well as valuable insight for the development of more advanced molecular robots that might be applied for the creation of artificial cell models, more intelligent drug delivery systems, and medical micro-robots, akin to white blood cells. Thus, this study will pave the way for the creation of artificial molecular systems comparable to living systems and will open the new door for robotics at a molecular level.