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Author(s)	石井，さつき
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学 位 論 文 の 要 約

博士の専攻分野の名称　　博士（理学）　　氏名　　石井　さつき

学 位 論 文 題 名

Microtubule Swarm Programmed with *p-tert*-Butyl Substituted Azobenzene Tethered DNA
（*p-tert*-ブチル置換アゾベンゼン修飾 DNA によってプログラムされた微小管の集団運動）

Swarming is a collective behaviour displayed by moving or self-propelled objects purely by local interactions among them. In nature, a wide range of self-propelled objects such as animals, birds, cells, and bacteria exhibit swarming or other collective behaviours to construct fascinating large scale patterns. This allows the objects to obtain emergent functions such as parallelism, robustness, and flexibility which cannot be achieved by a single entity. In recent years, many attempts have been made to mimic swarming in the field of material science using micro-sized self-propelled materials to understnand the mechanism of such collective behaviours. In particular, natural biomolecular motor systems, microtubule (MT)-kinesin have emerged as ideal candidates for experimentally demonstrating collective behaviours, due to the small size and self-propelled ability, as well as the potential for a wide range of applications in the fiels of nanotechnology. However, until now, it was difficult to control the local interactions among the self-propelled biomolecular motors for the swarming. To overcome this problem, photoresponsive DNA (*p*DNA) conjugated MT was developed. Photo-regulated swarming of MTs were achieved previously by employing DNA which contains azobenzene as the photoswitch where the formation and dissociation of the swarm in response to Vis and UV light, respectively (Vis-ON swarm system). However, having only one type of photoswitch to control the swarming could potentially become a bottleneck, when trying to construct a further complicated system. In this dissertation, the main objective was to establish a new photo-regulated biomolecular motor-based swarm system for the expanded application in the field of nanotechnology.

In **chapter 1**, the purpose of this dissertation and the background of this study are described by reviewing the previous studies using collodal particles and stating the limitations of the previously established Vis-ON system.

In **chapter 2**, swarming of MTs after UV light irradiation and the dissociation into individual MTs upon visible light irradiation was demonstrated by employing *p-tert*-butyl azobenzene incorporated DNA. Entirely reversed control of swarming is from the previously reported system was achieved, where swarming was initiated with visible light irradiation and the dissociation upon UV light irradiation. Furthermore, the effect of UV light

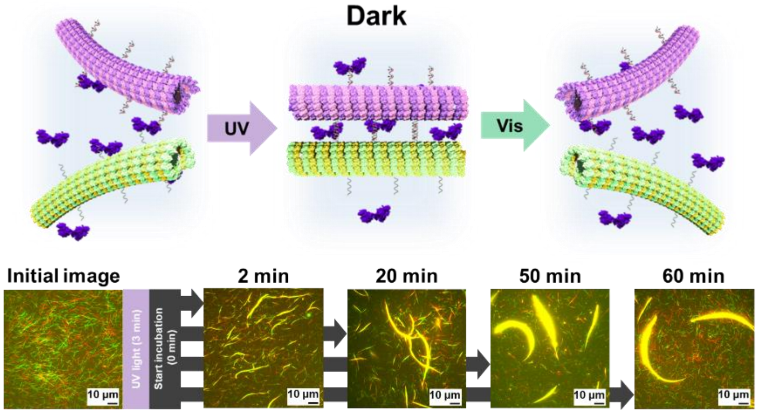

Figure 1. Scheme and fluorescence image of swarm formation and dissociation.

irradiation towards the swarm formation was systematically investigated, where necessity of "dark condition" after the UV light irradiation for the swarm formation was revealed.

Finally, the control of reversible swarming of MTs in a repeated manner by irradiating the MTs with alternate visible and UV light irradiation was also demonstrated.

In **chapter 3**, the novel behaviours of *p*DNA carrying *p-tert*-butyl azobenzene that was used for the photo-regulation of swarm under UV light irradiation is demonstrated by carrying out standard fluorescent quenching experiment and UV-Vis absorption measurement to evaluate a ratio of duplex formation. At the photo-stationary state (PSS) of UV light, the DNA formed the duplex with the complimentary DNA strand due to the *trans*-to-*cis* isomerisation of the azobenzene. However, unexpected discovery of the partial dissociation of the duplex into the single strands by the prolonged UV light irradiation after PSS was observed. It was revealed that, the equilibrium of the *p*DNA in single state and duplex is different when in ground state and PPS under the UV light irradiation. In addition, when the sample was incubated in dark after the UV light irradiation, it induced the duplex formation. Until now, the equilibrium of *p*DNA duplex formation was only evaluated by only enough UV light irradiation to achieve *cis*-to-*trans* isomerisation of azobenzene, hence this result indicated the possibility of a new photoswitch property of *p*DNA.

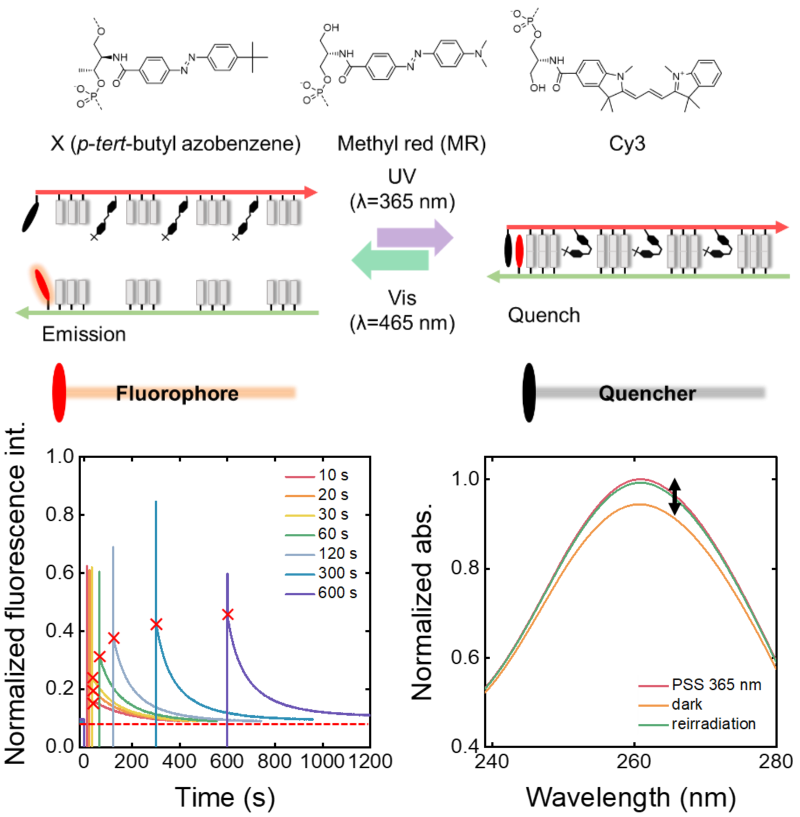

Figure 2. Schematic illustration of the experimental design (top), the result of time trace of fluorescence intensity measurement (bottom left), and the result of absorption measurement (bottom right).

In **chapter 4**, an orthogonal control of swarm system is achieved by integrating UV-ON and Vis-ON swarm systems in parallel to the same experimental system. The swarms were actively responded by associating and dissociating into individual MTs in response to the appropriate light signal. Until now, orthogonal, and reversible control was only demonstrated in static systems, hence this is the first report on the orthogonal control of the self-propelled biomolecular motor system where MTs can actively respond to the different light signals by emerging swarms or

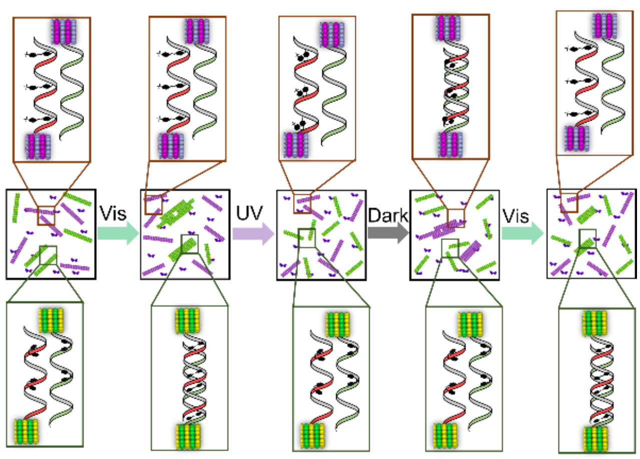

Figure 3. Scheme of orthogonal control of swarming in response to a light signal.

dissociating into individuals. This result is the first example which demonstrated the robustness, one of the emergent function obtained by collective behaviour.

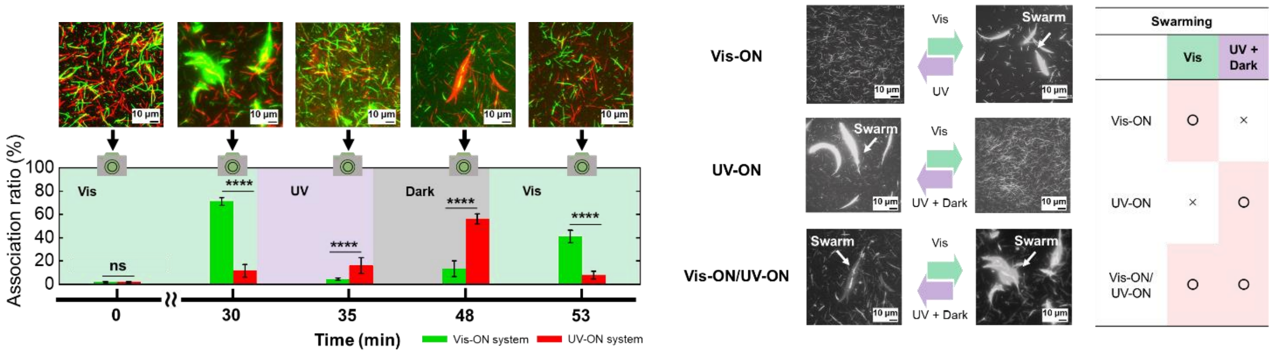

Figure 4. Fluorescence image of the changes of the swarming of Vis-ON and UV-ON system under the Vis and UV light irradiation (left), and prrof of robustness of the system (right).

In **chapter 5**, all the important result described in chapter 2 to 4 are summerised, which is establishment of new control method of biomolecular motor-based swarm system using *p*DNA, and the evaluation of the behaviour of *p*DNA under the UV light irradiation. Finally, future aspects of this research are stated as the concluding remarks.

This dissertation describes the establish a new photo-regulated biomolecular motor-based swarm system, where *para tert*-butyl azobenzene was incorporated into the DNA backbone as a photoswitch to regulate ON/OFF switching of the swarming. Introducing a new photoswitch incorporated DNA extend the programmability of the biomolecular motor system to expand the potential applications of biomolecular motors in many fields ranging from biotechnology to developing photo-regulated molecular machienes. Furthermore, the approach of introducing different photoswitches in the same system may contribute to the construction of further complex swarm systems for extended applications in molecular robotics.

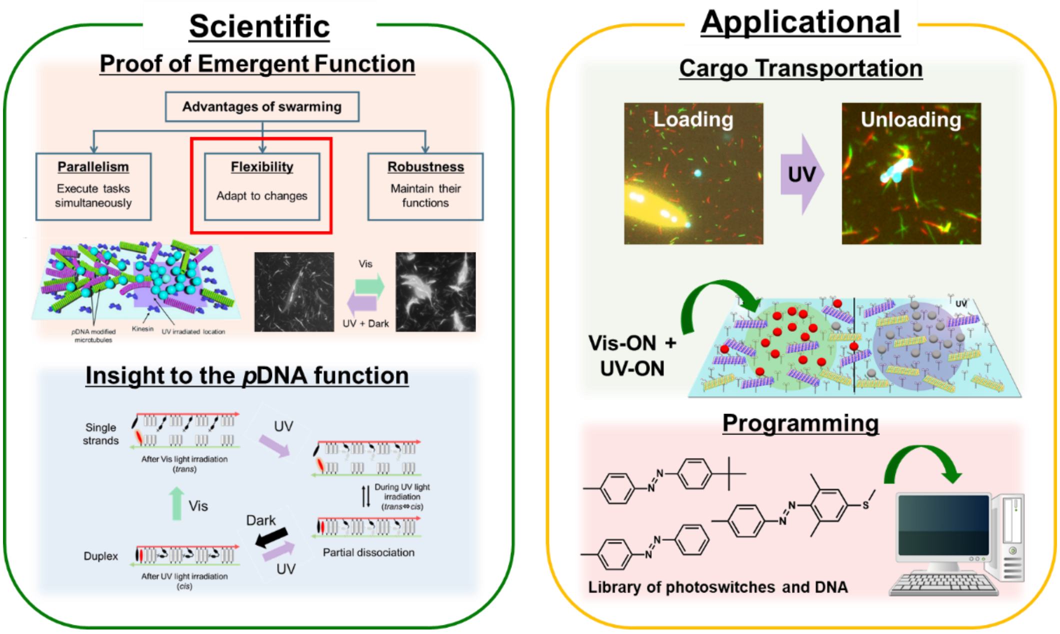

Figure 5. Future aspects of this research.