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Title	Active Self-Organization of Microtubules and Formation of Two-Dimensional DNA Network by Biomolecular Motor System [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(理学)
Dissertation Number	甲第14688号
Issue Date	2021-09-24
Doc URL	https://hdl.handle.net/2115/83271
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	Farhana_Afroze_abstract.pdf, 論文内容の要旨



学 位 論 文 内 容 の 要 旨
博士の専攻分野の名称 博士（理学） 氏名 アフロズ ファルハナ

学 位 論 文 題 名

Active Self-Organization of Microtubules and Formation of Two-Dimensional DNA Network by Biomolecular Motor System

(生体分子モーターシステムによる微小管の能動的自己組織化と2次元DNA ネットワークの形成)

Biomolecular motor systems have been attracted continuous interest as a machinery in the cell by converting chemical energy into mechanical work with high efficiency. In order to generate the power, for example, in the muscle, the self-organization of the biomolecular motor systems into the ordered structure should be required. Therefore, the control of the unidirectional motions, monopolar flocking, of the biomolecular motor systems by the self-organization is very important for the further applications of the biomolecular motors with respect to nanotechnology as well as the molecular robotics. On the other hand, in our group, recently the biomolecular motor system (microtubules-kinesin) has been conjugated with DNA (as processor) to self-organize MTs. Self-assembly of DNA under in vitro motility conditions of the biomolecular motor system provided swarm robots. It prompted me to use them together (biomolecular motor system and DNA) for organizing DNA in an ordered and complex network structure. In this dissertation, the unidirectional self-organization of MTs driven by kinesin and self-organization of DNA in a complex pattern mediated by biomolecular motor (MTs-kinesin) system have been summarized.

In **Chapter 1**, the purpose of the dissertation and background of the study have been explained.

In **Chapter 2**, the self-organization of the kinesin-driven microtubules was investigated as a kind of collective motion. In *in vitro* experimental system high density of microtubules (MTs) moving on the kinesin coated substrate generate the chiral rotating structure. From the experimental results and analysis, it was found that CCW rotational motion of MTs induced the spiral pattern formation in the collective motion, and above a certain density of MTs monopolar flocking generated. The unidirectional arrangement of the direction of collectively moving MTs was observed. This study will help to provide the information that how the chirality can affect the overall exhibited collective motion and large-scale pattern formation in the artificial systems.

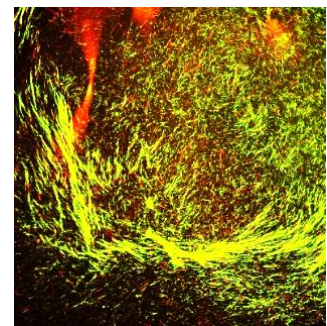


Figure 1: Unidirectional CCW rotational motion of high density MTs driven by kinesin

In **Chapter 3**, a new approach had been introduced to self- assemble large DNA into the network structure as an organized complex pattern by the biomolecular motor (MTs- kinesin) system. A primer and a template DNA were combined by ligation, which termed as ligated DNA. MTs were conjugated with this ligated DNA by click

reaction. Then the rolling circle amplification (RCA) was performed to yield MTs with the long strand elongated DNA. This elongated DNA conjugated MTs were subjected to in vitro motility assay, where DNA was straightened or tethered by the movement of MTs over the kinesin coated surface. The cross-linking of these tethered DNA with one another assembled themselves in the complex network structure. The formation of DNA network developed very rapidly due to the continuous movement of MTs. This study combines the RCA reaction of ligated DNA with the biomolecular motor system to yield the MTs with long DNA and they assembled into the network structure. It may provide an opportunity to explore a new tool in the field of active matter as well as in biomedical and environmental fields.

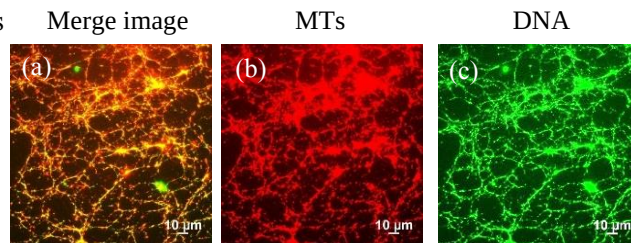


Figure 2: Fluorescence merge image of DNA network and MTs (a) MTs observed and (b) Amplified DNA network observed (c).

In **Chapter 4**, under the different physiochemical conditions such as concentration of DNA, incubation time for the amplification of DNA by RCA and density of microtubules, the formation of DNA network was investigated. Various control experiments were performed to verify the importance of biomolecular motor system (MTs-kinesin) and the gliding of MTs in the DNA network structure formation (self-organization of elongated DNA). These studies will help to provide necessary information to design and control large DNA network mediated by biomolecular motor system.

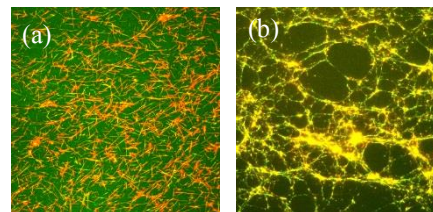


Figure 3: Without gliding of amplified DNA conjugated MTs, no DNA network can form (a); DNA network form by the gliding of amplified DNA conjugated MTs (b)

In **Chapter 5**, all the important results are summarized, and future perspectives have been described.

In this dissertation, the kinesin-propelled microtubules with the chiral structure can self-organize and exhibit rotational motion towards chiral rotating CCW direction, is described. A new method is described to self-organize long DNA in a complex network structure by in vitro gliding of elongated DNA conjugated MTs. The optimization of the physicochemical conditions to form the DNA network, is also investigated. The outcome of this dissertation approaches to the concept of programming the self-organization of cytoskeletal filaments by varying their density which provides us with a means to program the active-self organization of self-propelled particles. Moreover, the self-organization of long DNA by the gliding of cytoskeletal filaments, also will be helpful to widen the application in material science.