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Author(s)	伊藤, 正樹
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Mechanical Oscillation and MOF Transportation via Kinesin Powered Microtubules.

[Abstract]

Masaki ITO

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Graduate School of Chemical Sciences and Engineering

Hokkaido University, Japan

Contents

Purpose of This Dissertation..1

Outline of this Dissertation..2

Summary of Chapter 2...3

Summary of Chapter 3...4

Summary of Chapter 4...5

Purpose of This Dissertation

Self-organization in nonequilibrium state is a common process in living organisms in which chemical energy was consumed as time to construct complex emergent structures. On the other hand, it is one of the goals for material chemists to develop such system with non- living components for the sake of learning the strategies of how nature regulate complex chemical chain reactions and then adopting the theory into artificial world. Due to the recent development of biotechnology or deep insight for understanding biomacromolecules, it became more ease to handling biomacromolecules and utilizing these molecules as building blocks for constructing functional materials is an emergent topic in material sciences. One bio-synthetically approach for achieving non-equilibrium state outside of the cell, is to utilize motor protein which consume chemical energy to produce mechanical work for physical transformation as a building block of material construction. Here, the main issue of this dissertation is to develop the motor-protein based functional materials in non-equilibrium states and to understand the behavior of emergent structures.

Outline of this Dissertation

This dissertation is composed of four chapters in total including general introduction and concluding remarks to describe two approaches for constructing motor protein based self-assembled structures.

In Chapter 1, the purpose and the composition of this dissertation were described from the background of motor proteins. Chapter 2 demonstrated the construction of motile crystalline material through non-covalent interaction during the energy consuming process of motor protein system. The process was achieved by integration of metal-organic frameworks (MOF), which is a functional crystal composed of metal ion and organic ligand, and molecular motor protein system as power-generating modules. Through the post-synthetic modification (PSM) process, in which chemical modification on MOF was carried out after crystallization, MOF with biotin moiety on its framework was developed. PSM granted MOF not only ability to form composite but also significant water resistance which is sufficient for utilizing MOFs in high molarity aqueous media. Hybridization between MOF and MTs in the energy consuming motility assay was achieved by utilizing specific interaction of biotin-streptavidin bindings. The motile manner of the hybrid was obviously distinguished from that of MOF without chemical energy in the system. Chapter 3 focused on the collective behavior and dynamic shape transition of ring shaped assemblies of large numbers of MT, termed as MT rings, formed through active self-organization process of MTs which possessed under an air-buffer interface. As a result of the energy dispersive self-assembly process, collective motion of MT rings which composed of multiple MT filaments was exhibited with autonomous oscillation manifested through periodic changes in the size and shape with certain frequency. By comparing the time dependent behaviors of oscillation and deformation process of MT ring, accumulated stress which induced by the driving force of the motor protein system may one of the causes for the dynamic transition of the entire process. In Chapter 4, the knowledge revealed in this dissertation, the signification, and prospect for the future were mentioned.

Summary of Chapter 2.

Title of this chapter: Construction and Glidings of MOF-MT Conjugates

The development of active hybrid material was achieved by integration of metal-organic frameworks (MOF), which is a micron -sized functional crystal, and molecular motor protein system as power-generating modules. MOF was functionalized by modification of biotin moiety through the post-synthetic modification (PSM) method. Moreover, PSM enhances the water resistance of the MOF structure significantly to utilize in the high-molarity aqueous media. On the kinesin coated substrate, biotinylated microtubules (MTs) show kinesin-driven gliding motion in the presence of adenosine tri-phosphate (ATP) and was utilized as power transmission units which propagate the power stroke of kinesin motor proteins in order to move the conjugate forward. Attachment of MOF to MTs in the gliding motility geometry was achieved by specific interaction of biotin-streptavidin bindings. Time-lapse fluorescent microscopy revealed that MOF exhibit jittery Brownian motion under the static conditions, but in the presence of ATP, was allowed for translocation when gliding MTs collides with MOF and starts to power on it. The method demonstrates, for the first time, efficient preparation of bioconjugated-MOF in a non-equilibrium state and thus may constitute the key aspects of motor-driven hybridization.

Summary of Chapter 3.

Title of this chapter: Mechanical Oscillation of MT rings formed under an Air-Buffer Interface

During the initial process of tissue formation, living organisms regulate their size and shape by mechanical feedback, i.e. periodically-exerted mechanical external and/or internal stimuli modulate the shape of cell and even a whole structure of mass. Such fascinating self-organization phenomenon has never been achieved *in vitro* due to the complexity of interactions and inactiveness of the existing experimental system. Here, by using a molecular motor protein and a technique to confine the high-concentrated microtubules into 2D planner surface, emergence of collective motion of ring-shaped assemblies which composed of multiple MT filaments (MT rings) show mechanical feedback was obtained through energy dissipative self-assembly at an air-buffer interface. MT rings exhibit autonomous oscillation manifested through periodic changes in the size and shape. The oscillation of the MT rings came from attribution to a mechanical feedback arising from accumulated stress induced by the driving force of the motor protein system. The presented minimal feedback system may provide a new insight of constructing active materials or giving an insight into dynamic process in living systems.

Summary of Chapter 4.

Title of this chapter: Concluding Remarks

One of the goals for material chemists is to develop complex structure which is self-organized in nonequilibrium state composed of non-living components in the view point of learning the mechanism of how the natures modulate complex chemical chain reactions and then adopting the theory into artificial materials. Recent developments in biotechnology and understandings in biomacromolecules allow handling biomacromolecules as building blocks for constructing functional materials with remarkable features which originally comes from the bio-related modules. For developing nonequilibrium state in artificial systems, utilizing molecular motor protein which is also known as energy consuming nanomachine is regarded as one of the promising approaches. In order to develop organized material based on motor proteins, here two types of approach have described in Chapter 2 and Chapter3.

In Chapter 2, by utilizing strong interaction between biotin and streptavidin, construction of motile crystalline material during the energy consuming process of motor protein system was demonstrated. Combining motor protein and artificial material is one possible approach for the advanced application of motor protein outside of the cell. To this end, metal organic framework, which is a class of crystalline materials that consist of coordination bonds between transition-metal cations and multivalent organic linkers was selected as a conjugate material due to the possible heterogeneous modification onto the MOF framework. After following the post-synthetic modification (PSM) process to modify functional biotin moiety on the surface of MOF, the stability of MOF against soaking high-molarity aqueous solution was enhanced. This result providing an insight that PSM would widen the range of usage of MOFs which so far were recognized as instable in aqueous environment. Hybridization of MOF and MTs in the energy dispersive motility assay was achieved by utilizing specific interaction of biotin-streptavidin linkage. While developed method here for hybridization biotin-streptavidin interaction was used, this research suggests many avenues for future research. For example, adopting other adhesives enables to open the door for constructing assemblies. It will also be interesting to obtain motile hybrid MOF crystals which have stimuli-responsive chemical groups on its surface to control leaking of guest molecules.

In chapter 3, new approach to utilize motor protein system as a domain of active matter is developed by adopting air-buffer two-dimensional planner surface system in the motility assay of MTs was achieved in Chapter 3. Active matter is an upcoming concept in the field of natural science and computational physics to study the collective behaviors such as bird flocks and fish schools. Active matter systems are composed of energy consuming constituent components which drive the system to behave in very complex ways. These active materials have characteristic properties that are dramatically different from "hard" materials and have properties like self-motility, self-healing, internally generated flows, or synchronous dynamics. It is remarkable fact that the motor protein-based gliding system is the only approach to demonstrate the collective motion in molecular scale in vitro. In the chapter 3, focusing on the collective behavior and dynamic shape transition of MT rings composed of large numbers of MT filaments formed through active self-organization process of MTs which possessed under an air-buffer interface, while the entire structure of dense MT rings was seemingly stable, but some part of the structure is found to show dynamic shape transition. As the majority of the dynamic structures are MT rings which exhibited autonomous oscillation manifested through periodic changes in the size and shape with certain frequency, oscillation of MT ring is regarded as steady state of MT assemblies formed under an air-buffer interface. In the active systems reported previously, defects and turbulence which were quite resembled to MT rings were often recognized, but the mechanisms to create these structures were remained unsolved. In the case of MT rings, organization and reorganization process of MT rings was governed by mechanical feedback process which originated due to the mismatch in angular velocity at the inner and outer periphery of the MT rings. Moreover, these active systems can be also recognized as "active scaffoldings" for developing active hybrid materials. The behavior of these active systems remains to be explored for designing out of equilibrium behavior, with the goal of constructing new dynamic materials and systems.