Communications
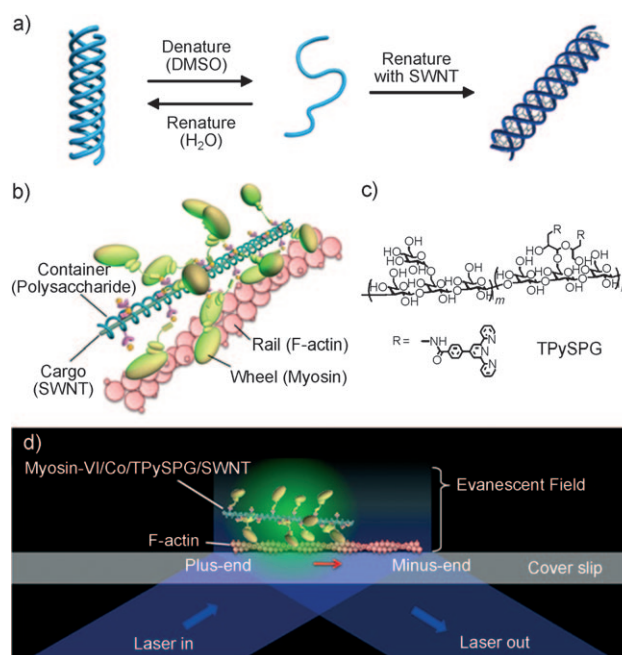
A Polysaccharide-Based Container Transportation System Powered by Molecular Motors**

Youichi Tsuchiya, Tomotaka Komori, Minako Hirano, Tomohiro Shiraki, Akira Kakugo, Toru Ide, Jian-Ping Gong, Sunao Yamada, Toshio Yanagida, and Seiji Shinkai

In living cells, the motor protein myosin, which is driven by ATP hydrolysis, intracellularly transports cargo such as vesicles and organelles[1,2] by moving along actin filaments. There have been many reports of how myosin can transport artificial cargoes such as polystyrene microspheres,[3,4] gold nanoparticles,[5] and quantum dots.[6,7] Recently, cargo transportation systems powered by artificial nanomotors were actively studied.[8–10] However, in these studies, the biomolecular or nanomotors are directly bound to the specific cargo. Therefore, these systems can be applied only to the delivery of certain types of cargo. Herein, we report the first container transportation system to be powered by biological motors. In this system, myosin is attached to a polysaccharide-based container that can hold a cargo. In fact, under physiological conditions, myosin binds to a container-like vesicle that holds a cargo.[2] As the polysaccharide can form complexes with various cargoes such as carbon nanotubes and DNA,[11] we envision that this novel container transportation system will expand the applicability of artificial intracellular transportation systems, including medically relevant procedures such as gene therapy.

We have previously reported the very interesting "dynamic" properties of β-1,3-glucan polysaccharides, which are typified by schizophyllan (SPG).[12] In nature, SPG adopts a triple-stranded helical structure (t-SPG), which dissociates into a single chain (s-SPG) upon dissolution in dimethyl sulfoxide (DMSO). The s-SPG chain can recover its original triple-stranded helix when DMSO is exchanged for water. These processes are referred to as denature (from t-SPG to s-SPG) and renature (from s-SPG to t-SPG), respectively (Figure 1a). We found that β-1,3-glucans and their derivatives can act as 1D hosts that helically wrap nanomaterials such as carbon nanotubes, conjugated polymers, DNA, and gold nanoparticles, and allow these nanomaterials to be dissolved in water through the denature–renature process.[11] Therefore, we selected SPG as the container for our system.

A schematic representation of our novel container transportation system is shown in Figure 1b. The "cargo" is wrapped with the "container" and transported on the "rail" by "wheels". We choose single-walled carbon nanotubes

[*] Dr. T. Shiraki, Prof. S. Shinkai
Nanotechnology Laboratory, Institute of Systems,
Information Technologies and Nanotechnologies (ISIT)
Nishi-ku, Fukuoka 819-0385 (Japan)
Fax: (+81) 92-805-3814
E-mail: shinkai_center@mail.cstm.kyushu-u.ac.jp
Homepage: http://www.isit.or.jp/index.en.html

Dr. Y. Tsuchiya[+]
Research Group for Molecular-informational Life Science, RIKEN
Hirosawa, Wako 351-0198 (Japan)

Dr. T. Komori,[+] M. Hirano, Dr. T. Ide, Prof. T. Yanagida
Graduate School of Frontier Biosciences, Osaka University
Yamadaoka, Suita 565-0871 (Japan)

Dr. A. Kakugo, Prof. J.-P. Gong
Division of Life Sciences, Faculty of Science, Hokkaido University
Kita-ku, Sapporo 060-0810 (Japan)

Prof. S. Yamada
Department of Chemistry and Biochemistry
Graduate School of Engineering, Kyushu University
Nishi-ku, Fukuoka 819-0395 (Japan)

Prof. S. Shinkai
Department of Nanoscience, Faculty of Engineering, Sojo University
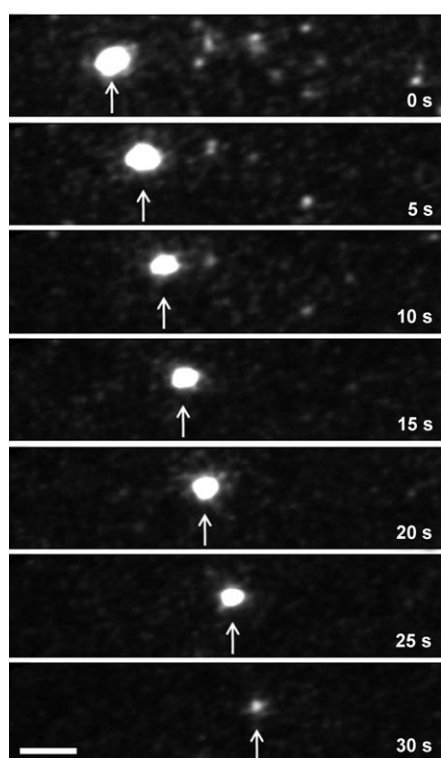Ikeda, Kumamoto 860-0082 (Japan)

[+] These authors contributed equally to this work.

Figure 1. a) Inclusion of SWNT by SPG by utilizing the renaturing process. b) Concept of the container transportation system for nanomaterials. c) Molecular structure of TPySPG. One of two possible cleavage forms is shown. d) Principle of TIRFM. Only fluorophores in the evanescent field, which is induced near the glass surface by the total reflection of excitation light, can be observed as fluorescent images.

(SWNTs) as the cargo since they can be wrapped with terpyridine-modified SPG (TPySPG, Figure 1 c). Recently, we reported that TPySPG shows 1D host properties and can coordinate ions much like a standard ligand.[13] Upon addition of Fe^{2+}, TPySPG can align SWNTs to form a sheetlike morphology. Since the terpyridine moiety can act as a strong ligand for metal ions,[14] we postulated that the terpyridine moieties on TPySPG can complex a protein to a nanomaterial by metal coordination. We used His-tagged, full-length myosin-VI labeled with green fluorescent protein [2,15,16] (GFP-myosin-VI-His_6; His = histidine) as wheels. His-tag is an oligohistidine and often exists as a hexamer (His_6) that can be used for protein affinity purification when using a metal chelate. His-tag has previously been used to attach proteins to a substrate.[17,18] Therefore, we investigated whether His-tag can facilitate proteins to complex with nanomaterials more efficiently.

Single-headed myosin-VI alone cannot move progressively along an actin filament (F-actin).[19] Rather, it must be dimeric or bound to a cargo.[20,21] Therefore, we can safely assume that all moving fluorescent spots are derived from GFP-myosin-VI bound to TPySPG/SWNT. Figure 2 (and Movie S1 in the Supporting Information) shows sequential total internal reflection fluorescence microscopy images (TIRFM, principle shown in Figure 1 d) of a myosin-VI coated TPySPG/SWNT (GFP-myosin-VI-His_6/Co/TPySPG/SWNT) composite (large bright spot) moving from left to right. The small, 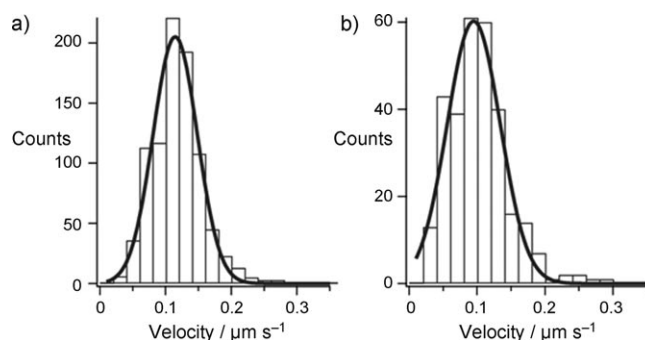faint, and immobile fluorescent spots are uncomplexed single-molecule myosin-VI. The composite traveled about 5 μm on an F-actin in 30 s. This result raises three important points. First, the enzymatic activity of myosin-VI ATP hydrolysis occurred after complexation with a Co^{2+}-modified TPySPG/SWNT (Co/TPySPG/SWNT) composite. Generally, myosin-VI tends to adsorb nonspecifically to many kinds of materials. For example, mixing myosin-VI and SWNT dispersions that lack TPySPG led to aggregates. However, these aggregates did not move, thus implying that nonspecific myosin-VI binding leads to loss of function. In contrast, the mixtures of miosin-VI with SPG/SWNT or with TPySPG in the absence of Co^{2+} did not result in fluorescent aggregates. This finding indicates that TPySPG acts not only as the linker but also as a protective layer to avoid nonspecific binding by myosin-VI. In addition, the Co^{2+}-modified terpyridine unit acts as a connector for the His-tag on myosin-VI. The fact that TPySPG acts as a linker is also supported by the observation that no fluorescent aggregates were formed for flag-tagged GFP labeled myosin-VI, which has no His-tag, when incubated with Co/TPySPG/SWNT (data not shown).

The second important point from our velocity observations is that the composite underwent long-range movements. The mean run length of dimerized myosin-VI is about 0.25 μm,[20,21] while our composite moved about 5 μm. This long-range movement is likely due to several myosins interacting with the SWNT. Because of this "cluster effect", the SWNT was able to enhance the motor function of myosin-VI. The third point is that this is the first report to show an artificial container transportation system that incorporates a molecular assembling method and utilizes coordination and noncovalent bonds. We have already reported how various nanomaterials can be complexed with SPG derivatives.[12] This report is the next step toward the transport of these complexes inside a cell and therefore has potential for broad applications including medical therapies.

Figure 3 shows the distribution of the GFP-myosin-VI-His_6/Co/TPySPG/SWNT composite velocity along an actin filament. The mean velocity of the composite was 95 nm s^{-1}, while that of control, which was determined by measuring the actin gliding movement over myosin-VI immobilized on the nitrocellulose coated glass surface,[22–24] was 110 nm s^{-1}, which

Figure 2. Movement of a GFP-myosin-VI-His_6/Co/TPySPG/SWNT composite (large bright point indicated by arrows) from left to right. Images were captured every 5 s from a TIRFM movie. Excitation: 488 nm laser. Scale bar: 2 μm.

Figure 3. a) Histogram of actin gliding velocity on myosin-VI. The solid line indicates a Gaussian function fit with a mean ± standard deviation of (0.110 ± 0.034) μm s^{-1}, $n = 890$. b) A histogram of GFP-myosin-VI-His_6/Co/TPySPG/SWNT complex velocities with a mean ± standard deviation of (0.095 ± 0.039) μm s^{-1} velocity ($n = 300$).

is consistent with a previous report.[24] The difference in velocity between the container transportation system and the control was negligible, meaning that myosin-VI can function as a motor protein even when complexed with Co/TPySPG/SWNT. Therefore, we concluded that Co^{2+} modified TPySPG is an ideal platform for His-tagged proteins.

We observed two remarkable composite movements. The first was a "landing-like" movement that occurred in the presence of a single actin filament. Figure 4 (and Movie S2 in the Supporting Information) shows sequential TIRFM images
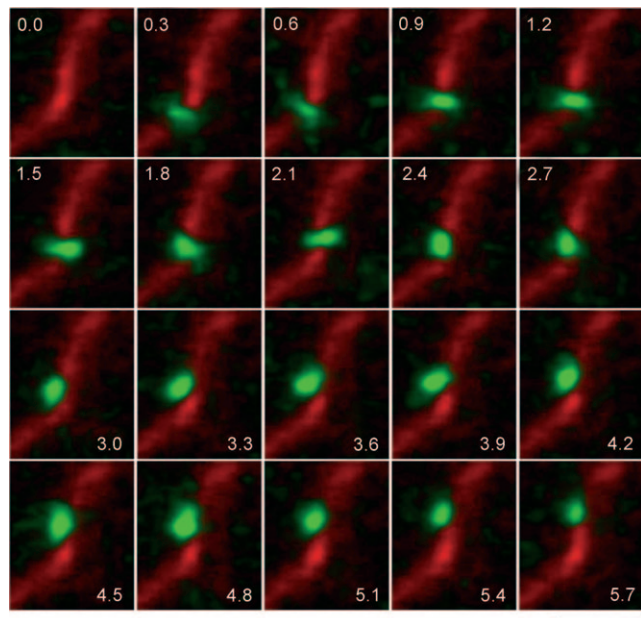
Figure 4. "Landing" of a GFP-myosin-VI-His$_6$/Co/TPySPG/SWNT composite (green) on F-actin (red). Images were captured every 0.3 s from a TIRFM movie. Excitation: 488 nm laser. Scale bar: 2 μm.

of the composite that just begins to move along the F-actin. Immediately upon interacting with F-actin, the composite moved with fluctuation, but was stabilized shortly afterwards. This observation demonstrates that GFP-tagged myosins interacted with the rigid rodlike structure of the TPySPG/SWNT composite. The second movement occurred in the presence of crosslinked actin filaments where the composite was able to switch filaments at the crosslink point (Movie S3 in the Supporting Information). At the first filament switch, the composite was unstable and appeared to detach. However, it promptly reattached to the adjacent filament and continued normal motility. At the second crosslink point, the composite smoothly underwent the switch. However, smooth switches only occurred in multi-myosin systems. Such behavior would be practical inside a cell since actin filaments exist as a meshwork and therefore are regularly crosslinked.[25] This property further demonstrates that the new system can function as an intracellular container-transportation system.

We have presented a novel cargo transportation system that uses a polysaccharide as the container. We have previously reported that β-1,3-glucans containing artificial derivatives have the flexible host properties needed for various nanomaterials.[12] Our results suggest that various nanomaterials complexed with β-1,3-glucans can be transported by this actomyosin–polysaccharide container transportation system.

We are currently developing new applications for this system and are specifically targeting gene therapy because F-actin aligns itself in a cell so that the plus-end is oriented toward the cell membrane while the minus-end is orientated toward the nucleus.[2] Since myosin-VI moves from the plus- to the minus-end, our transporting system could potentially transport a cargo to the nucleus. Furthermore, the combination of SPG and a myosin-VI based mobility system is promising not only for gene therapy but also for new nano- or microdevices[26, 27] and new molecular information communication systems.[28]

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