COLOCALIZATION OF Q-DOTS CARRIED BY MOTOR PROTEINS ON MICROTUBULE ARRAY IN NANOTRACKS

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ABSTRACT

We developed a molecular system to visualize molecular bindings as the colocalization of two Q-dots carried by motor proteins on a microtubule (MT) array with predefined polarities on nanotracks. One of the molecular systems, GSH-Q-dot525-dynein (GSH-Q525-D) and GST-Q-dot655-kinesin (GST-Q655-K), resulted in colocalizations by GST-GSH bindings. The other system, avidin-Q525-D (avi-Q525-D) and biotin-Q655-K (bio-Q655-K), also provided specific bindings of avidin-biotin. Analysis of run length (RL) and velocity showed molecular transport without losing motilities of motor proteins. Quantitative analysis proved that colocalizations were achieved by the designed molecules carried by motors. The result implies that an applicability of the reconstructed molecular system to further miniaturization of μTAS to a countable molecular scale, i.e. Molecular Total Analysis Systems (MTAS).

KEYWORDS: Motor protein, Kinesin, Dynein, Microtubule, Nanotransport, Molecular reaction

INTRODUCTION

For the past decade, the field of micro/nano fluidics or μTAS has been progressed based on micro fabrication technologies. Micro-scale fluidic devices downsized various conventional bench-top chemical synthesis or analysis to the on-chip scale. In order to advance further miniaturization to nanometer scale, a novel method for directly manipulating molecules is necessary because of larger pressure drop in nanofluidic channels. Since motor proteins have unique characteristics that include converting chemical potential energy to mechanical force, the proteins are regarded as power sources at the nanometer scale by combining with micro/nano fabrication technologies. So far, most of researches utilize MTs on motor proteins as a shuttle of cargo molecules because multiple motors stably support the MT motility in the assay [1]. However, the other motor protein-based transport, in which motors carry cargos on MTs, has an advantage: each cargo can be directly carried by motors that is the equivalent molecular configuration with the intracellular transport [2]. Taking this advantage, we construct a molecular manipulation and reaction system, a demonstration of MTAS, driven by motor protein, kinesin and dynein, as transporters on a MT array.

EXPERIMENTS

Orientation of MT polarities: Kinesin moves on MTs from minus end to plus end and dynein moves reversely. Because of the directed motility, polarity-oriented MTs on nanotoracks array are applicable for regulating the direction of molecular transport. Figure 1 shows schematics of orientation, MTs are supported by kinesin immobilized on surface and glide into nanotracks. The orientation of MT polarity was realized on nanotracks fabricated on a coverslip coated with 150-nm Al and 150-nm ZEP 520A resist by electron beam lithography. Over 90% of MTs were oriented in the same direction by utilizing gliding of MTs in 400-nm-width track and MT dissociation method at one end of tracks. Then, immobilization of MTs was achieved by glutaradehyde with maintaining functionality of motor proteins [3].

Design of molecular system: We designed two pairs of cargo molecules which have affinities by molecular specific bindings. One of them was GSH-Q525-D and GST-Q655-K for GSH-GST binding, using biotin-PEG-GSH

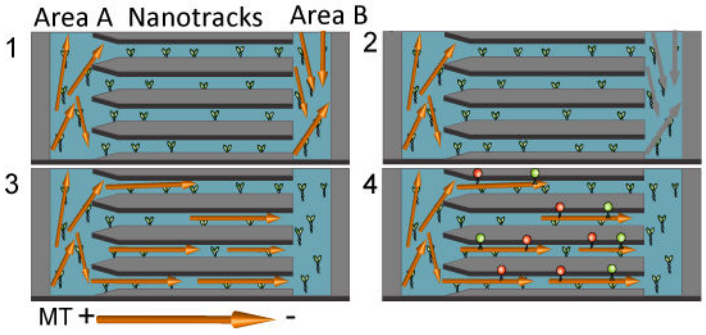

Fig. 1: Schematics of preparation and usage of the microtubule (MTs) arary. 1) Kinesin motors were immobilized on the Pluronic-treated glass surface, and MTs were introduced and immobilized on kinesin. 2) Area B was treated with the MT dissociation method to remove MTs. The method enabled MTs to enter nanotracks from area A only, resulting in orientation of MT polarities. 3) When ATP solution was introduced in the flow cell, MTs started gliding into nanotracks from area A. Once the optimized assay time (10 min) was elapsed, the MT array was immobilized with aldehyde. 4) Kinesin-labeled Q-dot 525 and dynein-labeled Q-dot 655 were carried toward the plus end and minus end of MTs, respectively.

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molecule and biotinylated GFP-GST protein (Fig. 2a). The other set was avi-Q525-D and bio-Q655-K for avidin-biotin bindings (Fig. 2b), where biotin-PEG-biotin molecule and streptavidin-coated Q-dots were used. Specificity of bindings were evaluated by observing colocalizations of Q-dots after mixing in two solutions containing GSH-Q525-D and GST-Q655-K, or avi-Q525-D and bio-Q655-K. The bindings were also observed by sequentially introducing to a glass flow cell.

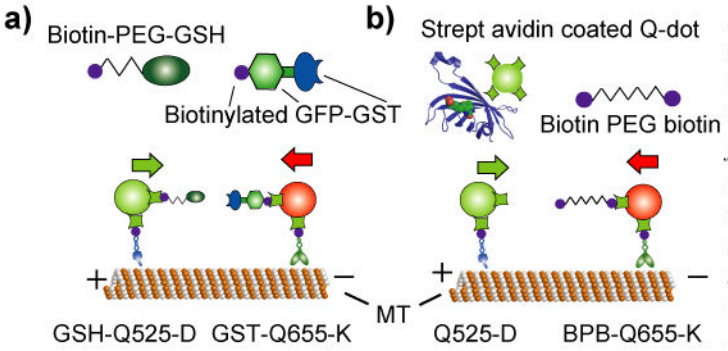

Fig. 2: Two kinds of molecule pairs used for run length (RL), velocity and binding measurements. a) GSH (glutathione) and GST (glutathione -S - transferase) pair. Both GSH and GST are functionalized with biotin, and immobilized on dynein - labeled Q-dot 525 (GSH-Q525-D) and kinesin-labeled Q-dot 655 (GST-Q655-K), respectively. b) Avidin and biotin pair. Streptavidin-labeled Q-dot 525 is carried by dynein (avi-Q525- D).Bioitin-PEG-biotin (BPB) is carried by kinesin-coated Q-dot 655 (bio-Q655-K). The full molecular complexes were assayed on the MT.

Colocalization of Q-dots and motility evaluation: Two pairs of molecules were assayed on the MT array to visualize colocalization of Q-dots caused by specific bindings of molecules. For GST-GSH system, we prepared full molecular complexes, GSH-Q525-D by conjugating the Q-dot 525 (10 nM) and biotin-PEG-GSH (25 nM) and then adding dynein (100 nM). Each molecule was conjugated by incubating the solution on ice for 15 minutes. Another complex, GST-Q655-K was prepared by the same protocol using biotinylated GFP-GST and kinesin. Both GSH-Q525-D and GST-Q655-K were diluted to 2 nM in ATP solution (1 mM) and injected on the MT array. Two Q-dots moved in the opposite directions following kinesin and dynein motility on MTs, and eventually some of them passed each other or bound at their collision. Control experiment was implemented by eliminating biotin-PEG-GSH molecule from the full colocalization assay.

For avidin-biotin binding we prepared Q525-D and Q655-K. Then, we immobilized Q655-K on the MT array, and incubated biotin-PEG-biotin (500 nM) for 5 min sequentially. It is because premixing Q655-K and biotin-PEG-biotin to produce bio-Q655-K has potentially cause inhibition of avidin-biotin binding or kinesin-MT interaction. After that, excess molecules were eliminated and Q525-D (2 nM) was injected. Colocalization of Q-dots was observed as for the GST-GSH binding. Control experiment was implemented by eliminating the biotin-PEG-biotin molecule.

In addition to the colocalization experiments, we measured velocities and run lengths (RL) of the molecular complexes, because it was suspected that designed molecules might disturb motility of motors.

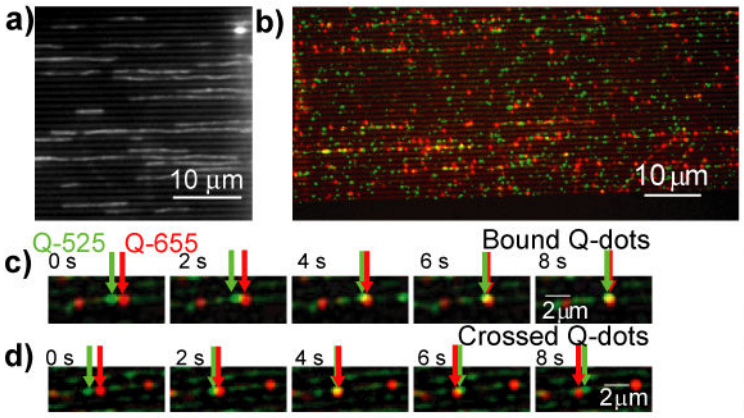

Fig. 3: a) The MT array in nanotracks (w = 400 nm, space = 1 mm). Polarities are oriented with the orientation ratio of over 90% by applying the dissociation method. b) Bidirectional transport of the full molecular complexes on the MT array. Q-dots represented by red spot (GST-Q655-K) and green spot (GSH-Q525-D) move according to their motor motilities. c) Sequential images of Q-dots bound by their molecular specific binding. d) Q-dots crossed without binding each other. Numbers of bound or crossed Q-dots were counted and compared with control experiments without binding molecules.

RESULTS AND DISCUSSIONS

The MT array was prepared on nanotracks as shown in Fig. 3a. In Fig. 3b, green and red spots represent GSH-Q525-D and GST-Q655-K, respectively, and they are simultaneously observed by DualView optical system. Focusing on individual Q-dots, we were able to identify binding or crossing of two Q-dots while moving on MTs. Figures 3c and 3d are the sequential images of passing and binding Q-dots. In Fig. 3c, green and red Q-dots moved in the opposite direction, and finally resulted in a colocalization, while, in Fig. 3d, two Q-dots passed each other.

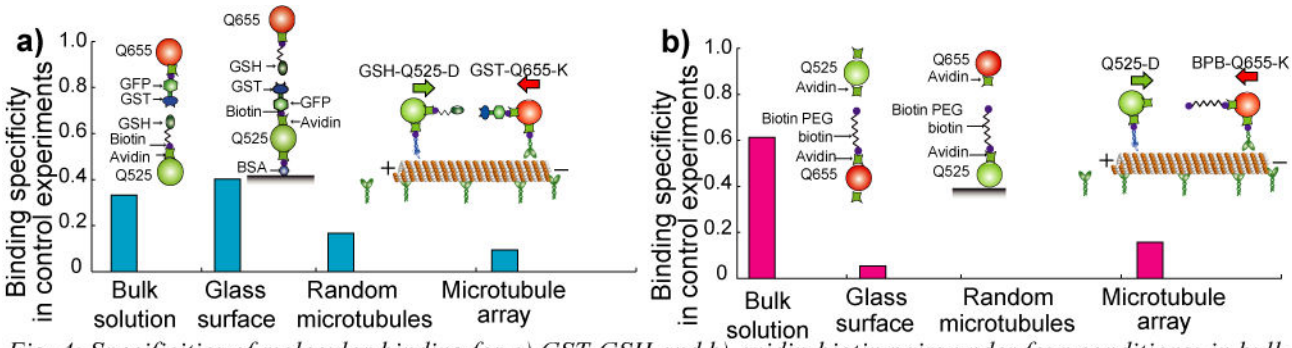

Fig. 4: Specificities of molecular binding for a) GST-GSH and b) avidin-biotin pairs under four conditions: in bulk solution (w/o motor), on glass surface (w/o), MTs immobilized randomly (w/motors), and on the MT array (w/motors).

For the evaluation of colocalizations on the MT array, we counted the number of passing and binding of Q-dots, and calculated an indicator: binding specificity in control experiments (Fig. 5). Results in control experiments are normalized by those in the experiments using the colocalization assay. Normalized binding specificity means that the lower values indicate higher specificity in the colocalization assay. Results indicate that Q-dot colocalizations were caused depending on specific bindings of GST-GSH or avidin-biotin. In the case of GST-GSH, specificity on MTs was higher than that seen without motors in a bulk solution or on a glass surface. And in the case of avidin-biotin, low specificity in a bulk solution was obtained. It is presumably because two biotin moieties in biotin-PEG-biotin bound to the surface of a Q-dot.

We also measured velocity and RL of each complex. RL of GST-Q655-K was 4.4 μm and velocity was 0.32 ± 0.10 μm/s (Fig. 4a). GST-Q655-K had comparable velocity and RL to Q655-K. The same comparison was done between Q655-D and GSH-Q655-D (Fig. 4b), and between Q655-K and bio-Q655-K (Fig. 4c). Though RL of GSH-Q655-D was 3.9 μm, decreased by 29% compared to Q655-D, velocity was comparable (0.24 ± 0.10 μm/s for GSH-Q655-D, 0.22 ± 0.10 μm/s for Q655-D). RL and velocity didn't show significant differences between bio-Q655-K and Q655-K. These results indicate that the designed molecules did not deteriorate motility of motors.

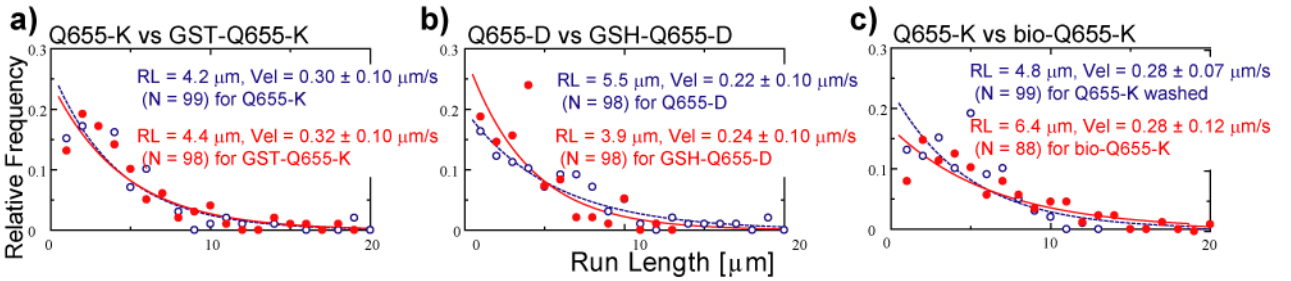

Fig. 5: Distributions of RL are fitted with an exponential decay curve to derive average RLs. Velocities are shown as average ± S.D. The results prove motilities of motors are preserved with molecular complexities.

CONCLUSION

In this paper, we demonstrated an experimental system of molecular transport and visualization of molecular bindings driven by motor proteins. Molecular transport by kinesin and dynein on the MT array on nanotracks was established using newly designed molecules. In addition, designed molecule pairs showed colocalization of Q-dots on the MT array. Our results indicate feasibility of molecular manipulation and reactions driven by kinesin and dynein motors *in vitro*. This is the first step toward miniaturizing μTAS to nanometer scale, which can be considered as MTAS.

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