

## Electronic Supplementary Informations

### METHODS

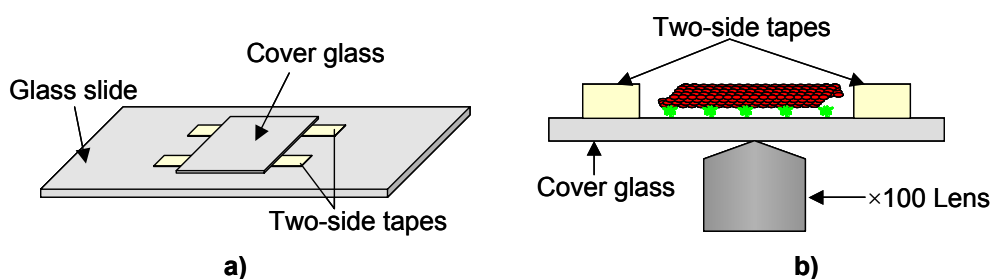
**Microtubules and kinesin.** Tubulin was purified from pig brain and was labeled with rhodamine or biotin.<sup>1</sup> The rhodamine-labeled and biotinylated MTs were obtained by copolymerization of rhodamine-labeled tubulin (2.5  $\mu$ L, 1.3 mg/mL in PME 80 buffer) and biotinylated tubulin (2.5  $\mu$ L, 1.3 mg/mL in PME 80 buffer) at 37 °C for 40 min in the presence of 60 % glycerol-PME 80 buffer [1  $\mu$ L, 80 mM potassium PIPES (pH 7.4) containing 1 mM MgCl<sub>2</sub>, 2 mM EGTA (O,O'-bis(2-aminoethyl)ethylene glycol-N,N,N',N'-teraacetic acid), 60 % glycerol, and 6 mM GTP]. Distribution of the biotinylated tubulin in the resultant MTs was observed by streptavidin-Alexa Fluor 488 (Ex: 494 nm, Em: 518 nm; Molecular Probes, Inc. Eugene, Oregon, USA). As a result, it was confirmed that the rhodamine-labeled tubulin and the biotinylated tubulin were evenly distributed along the MTs. After the polymerization, taxol (0.1  $\mu$ L, 1 mM in dimethyl sulfoxide) was added to stabilize the formed MTs. Kinesin was prepared from *Escherichia coli* expressing recombinant protein K 560, dimeric human conventional kinesin with C-terminal truncation.<sup>2</sup> The concentration of purified kinesin was 1.5 mg/mL.

**Binding of hetero-bifunctional latex particles with microtubules.** The preparation of biotinylated and PK-immobilized hetero-bifunctional latex particles and the characterization of immobilized PK were described in the previous paper.<sup>16,17</sup> For preparing the hetero-bifunctional latex particles modified by a biotin derivative containing polyethylene glycol (PEG) segment, biotin-PEG-CO<sub>2</sub>-NHS (Shearwater Polymers, Inc.; average Mw: 3400) was used instead of 5-(*n*-succinimidyl)oxycarbonyl)pentyl D-biotinamide (Biotin-X-NHS). The amount of biotin-PEG-CO<sub>2</sub>-NHS was about 200-fold molar excess to the hydroxyl groups on the particle surface. The remaining unreacted epoxy groups of the particles were treated with excess aminoethanol to prevent reactions between the epoxy groups and streptavidin and/or MT. PK immobilized on the latex particles were then labeled with equivalent mole of BODIPY FL-X

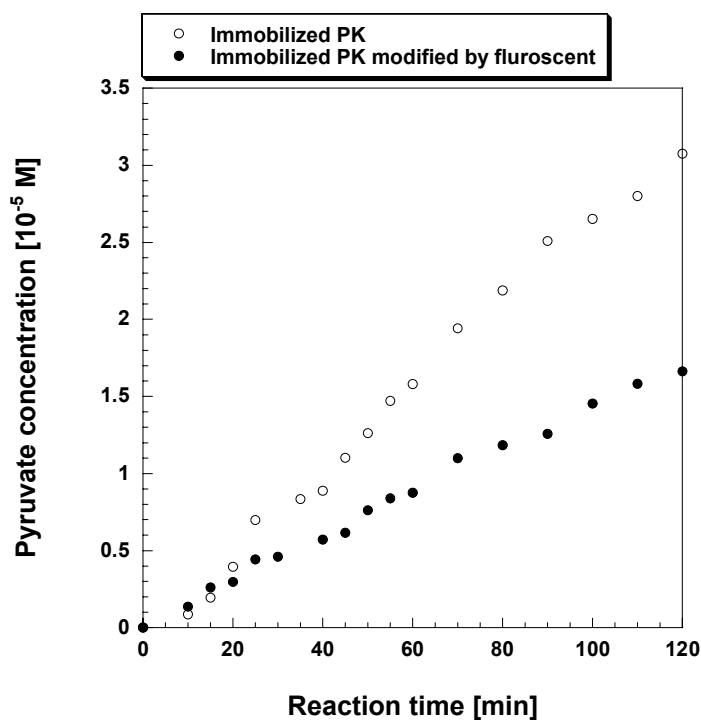
[6-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino)hexanoic acid succinimidyl ester] (Ex: 504 nm, Em: 508 nm, Molecular Probes, Inc. Eugene, Oregon, USA), as follows. After the suspension (1 mL) of the biotinylated and PK-immobilized particles (particle weight: 0.015 g) were centrifuged, the pelleted particles were resuspended in 1 mL phosphate buffer (PB, pH 7.4), BODIPY FL-X solution (2  $\mu$ L, 1 mg/mL in dimethyl sulfoxide) was added and the reaction was conducted at room temperature for 2 h with continuous rotation. After the reaction, the latex particles were washed three times with PB (pH 7.4) with the help of centrifugation and ultrasonication, and were then resuspended in 1 mL PB (pH 7.4). Equimolar streptavidin (25  $\mu$ L, 1 mg/mL solution, Wako Pure Chemical Industry Co. Ltd. Japan) was further introduced to bind with the biotin sites with continuous rotation at room temperature for 2 h. After streptavidin was attached to the biotin sites, the particles were washed three times with PB (pH 7.4) with the help of centrifugation and ultrasonication, and were then resuspended in 1 mL assay buffer (10 mM Tris-acetate (pH 7.5), 50 mM potassium acetate, 2.5 mM EGTA, 4 mM magnesium sulfate) containing 20  $\mu$ M taxol. For combining the particle with MTs, MT solution (0.5  $\mu$ L) was diluted by assay buffer (200  $\mu$ L) containing 20  $\mu$ M taxol and then the solution was mixed with assay buffer (200  $\mu$ L). Binding of the particles with the rhodamine-labeled and biotinylated MTs was performed at room temperature for various reaction times (2–10 min). In this range of reaction time, no undesirable multiple aggregation occurred between MTs and the particles and no difference exists in the binding. To remove the inhibitory effect of particles on combining MTs with kinesin, the particles were bound to MTs after the MTs were coupled with kinesin on the glass surface.

**Motility assay.** Motion of MTs bearing the PK-immobilized particles (MT-particle complex) was observed on kinesin-coated glass surface in the flow chamber (Fig. 3) by a fluorescence microscope (IX 70, Olympus, Tokyo, Japan) following standard protocols<sup>8</sup> with several minor modifications. Briefly, the flow chamber was constructed with a cover glass, a glass slide and double-sided tapes. Solution A

was prepared from assay buffer (1 mL), 2-mercaptoethanol (2  $\mu\text{L}$ ), and casein (50  $\mu\text{L}$ , 10 mg/mL in Tris-HCl (pH8.0)). Kinesin solution (4  $\mu\text{L}$ ) was diluted with solution A (100  $\mu\text{L}$ ). This kinesin solution (20  $\mu\text{L}$ ) was poured into the flow chamber and kinesin was immobilized on the glass surfaces (2 min). Solution B was prepared from assay buffer (1 mL), 2-mercaptoethanol (2  $\mu\text{L}$ ), taxol (2  $\mu\text{L}$ , 10 mM), and casein (50  $\mu\text{L}$ , 10 mg/mL in Tris-HCl (pH8.0)). After the flow chamber was washed three times by solution A, MT solution (20  $\mu\text{L}$ ) prepared by diluting MTs (0.5  $\mu\text{L}$ ) with solution B (200  $\mu\text{L}$ ) was poured into the flow chamber and MTs were allowed to bind to kinesin on the glass surface (2 min). Unbound MTs were removed by washing three times with solution B. Solution C was prepared from assay buffer (1 mL), 2-mercaptoethanol (2  $\mu\text{L}$ ), taxol (2  $\mu\text{L}$ , 10 mM), casein (50  $\mu\text{L}$ , 10 mg/mL in Tris-HCl (pH8.0)), glucose (10  $\mu\text{L}$ , 300 mg/mL), catalase (10  $\mu\text{L}$ , 2 mg/mL), and glucose oxidase (10  $\mu\text{L}$ , 10 mg/mL). In the case of MTs without the particles, solution C (20  $\mu\text{L}$ ) containing various ATP concentrations was introduced into the flow chamber. To prepare MT-particles complex, on the other hand, 20  $\mu\text{L}$  particle suspension (prepared by suspending 1.5 mg particles into 500  $\mu\text{L}$  solution B) was poured in the flow chamber to allow the particles to bind with MTs (5 min). After the flow chamber was washed three times with solution B, solution C (20  $\mu\text{L}$ ) containing ADP (1 mM), phosphoenolpyruvate (PEP, 1 mM), KCl (25 mM), and  $\text{MgCl}_2$  (6 mM) was introduced into the flow chamber. All the fluorescence observations were carried out at room temperature (ca. 25  $^\circ\text{C}$ ).



**Figure 1.** (a) Flow chamber and (b) its cross-section for fluorescence observation.



**Figure 2.** Time course formation of pyruvate by PK (labeled by BODIPY FL-X) immobilized on the beads: 0.1 mM ADP, 0.1 mM PEP, 0.11 mg immobilized PK/L, room temperature. The amount of immobilized PK was obtained according to references 16 and 17.

**Video 1.** Motion of fluorescence-labeled MT-particle complex powered by self-supplied ATP in the flow chamber. The motion is shown by accelerating it 400 times.

**Video 2.** Motion of fluorescence-labeled MT-particle complex powered by ATP (1 mM) supplied from outside. The motion is shown by accelerating it 4 times.

**References:**

1. A. A. Hyman, D. N. Drechsel, D. Kellog, S. Salser, K. Sawin, P. Steffen, L. Wordeman, T. Mitchison, *Methods Enzymol.* 1991, **196**, 478-485.
2. G. Woehlke, A. K. Rubby, C. L. Hart, B. Ly, N. Hom-Booher, R. D. Vale, *Cell* 1997, **90**, 207-216.