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Supporting information

Accurate polarity control and parallel alignment of actin filaments for myosin-powered transport systems

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1. Protein preparation

Actin was purified from rabbit skeletal muscle,[1] and a fraction of purified actin was biotinylated by Sulfo-NHS-biotin (341-06801, Dojindo).[2] Myosin V was purified from chick brains.[3] The expression system of the ×6 His-tagged gelsolin mutant[4] was a kind gift from Y. Y. Toyoshima (The University of Tokyo, Japan). The mutant was expressed in *E.coli* and purified by His-tag affinity column (30450, Qiagen). Biotinylated-casein was prepared by reacting Sulfo-NHS-biotin (341-06801, Dojindo) to α-casein (C-8032, Sigma-aldrich) in a 10:1 molar ratio and purified by gel chromatography (AKTA explorer 10, Superdex 200 HR 10/30; GE Healthcare).

2. Buffer

All experiments were performed in F buffer (10 mM Imidazole-HCl pH 7.6, 100 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA, 5 mM DTT). To observe the fluorescent image of actin filaments under an optical microscope, the oxygen scavenger system (216 μg ml^{-1} glucose oxdase, 36 μg ml^{-1} catalase, 4.5 mg ml^{-1} glucose, 1% β-mercaptoethanol) was added to F buffer in order to prevent scission and photobleaching of actin filaments.

3. Flowchamber

KOH-washed coverslips were prepared by rinsing coverslips (32 × 24 mm^2, 0.12–0.17 mm in thickness, NEO, Matsunami) with 0.2–1.0 M KOH in a bath sonicator for 30–60 min, and stored in distilled water at 4°C to prevent absorption of dust. The coverslip was air-dried just before use. Collodion-coated coverslips were prepared by dipping coverslips into 0.1% collodion (17-1002, Oken) in 3-methylbutyl acetate and the coverslips were air-dried. The flowchamber was assembled by placing two spacers of thickness 50 μm (2 × 20 mm^2 paper slips coated with silicon grease) on

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the KOH-washed or collodion-coated coverslips and untreated coverslip (18 × 18 mm^2, 0.12–0.17 mm in thickness, Matsunami) on top. The gap between the two spacers was ~5 mm.

4. Microscopy

We used an inverted microscope (IX70, Olympus) equipped with a stable sample stage (KS-O, Chuukousha Seisakujo) or a custom-build inverted microscope. In both cases, a ×100 oil iris objective lense (PlanApo NA 1.4, Olympus) was used. The fluorescent image was intensified (VS4-1845, Video Scope) and captured by a CCD camera (CCD-300-RCX, Dage-MTI), or the image was directly captured by a cooled EMCCD camera (MC681SPD-ROBO, Texas Instruments; iXon3 897, Andor Technology). The frame rate was 30 fps in all three cameras. All observations were done at 23–25 °C.

5. Preparation of gelsolin-capped actin filaments

To prepare gelsolin-capped actin filaments, the gelsolin mutant was mixed with both untreated and biotinylated actin monomers. The molar ratio between gelsolin, actin, and biotinylated actin, was 1 : 270 : 30. The mixed solution was kept on ice overnight for polymerization. Polymerized filaments were stabilized and stained with tetramethylrhodamine phalloidin (77418, Fluka) or rhodamine phalloidin (R415, Molecular Probes) to be visualized under a fluorencence microscope.

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6. Assembly process of iso-polar arrays of actin filaments

First, one volume (~15 μl; the inner volume of the flow chamber) of a solution of 1–2 mg ml^{-1} biotinylated-BSA (bovine serum albumin) (A8549, Sigma-aldrich) dissolved in F buffer was infused into the flowchamber and incubated for 1 min to coat the glass surface. Second, two volumes of 0.1–5 μM of biotinylated actin filaments terminated with gelsolin were injected and incubated for 3 min. Unbound filaments were washed out by 5–10 volumes of F buffer. Then, two volumes of 0.1–0.5 mg ml^{-1} streptavidin (21125, Pierce) dissolved in F buffer were flashed into the flowchamber to orient and immobilize the filaments in the downstream direction. Finally, unbound streptavidin was washed out by 5–10 volumes of F buffer. For the alternative method of surface treatment, the KOH-washed coverslip coated with 1–2 mg ml^{-1} biotinylated-BSA was replaced with either a collodion-coated coverslip coated with the same concentration of biotinylated-BSA or the KOH-washed coverslip coated with ~0.7 mg ml^{-1} biotinylated-casein. To anchor the gelsolin mustant through the histidine tag, a collodion-coated coverslip was coated with one volume of 200 μg ml^{-1} Anti-His_6 monoclonal antibody (11922416001, Roche) in PBS buffer for 3 min, followed by incubation with two volumes of 2 mg ml^{-1} casein (C5890, Sigma-aldrich) in F buffer for 1 min. For the fabrication of actin arrays by α-actinin, non-biotinylated actin filaments were used and 1–4 mg ml^{-1} of α-actinin (A9776, Sigma-aldrich) in F buffer was used instead of the streptavidin solution for the surface immobilization process.

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7. Preparation of myosin V-coated beads

First, the original bead suspension (L-5193, Molecular Probes; Latex fluospheres carboxylate-modified, ϕ = 0.01 μm, ex.580/em.605) was diluted 1000 times in F buffer and stored on ice. We sometimes found large aggregations of the beads. Such aggregations were removed by centrifugation (×11,000 g, 3 min). Just before use, the suspended beads were sonicated for several seconds to disperse aggregations of beads, and 2 μl of the suspension was mixed with 5 μl of F buffer, 1 μl of 2 M KCl (f. ~200 mM to attach myosin molecules to the beads in the active form [5]) and 2 μl of 38.5 μg ml^{-1} myosin V in this order (bead : myosin V = 1 : 3). The mixed solution was pipetted 30 times to minimize bead aggregation and incubated for 10 min on ice. Finally, 10 μl of 5 mg ml^{-1} BSA (A4378, Sigma-aldrich) was added and stored on ice for up to a week.

8. Myosin V motility assay

The iso-polar array of actin filaments was prepared in advance. Then, the myosin V-coated bead suspension was diluted twice in F buffer containing 1 mM ATP and the oxygen scavenger system and was infused into the flowchamber of the actin array. The beads suspended in the solution were stochastically bound to the immobilized actin filaments by their Brownian motion. The fluorescence intensity of the beads was much higher than that of actin filaments (Figure 4). Thus, the beads could be easily distinguished from the actin filaments. The myosin V-coated bound beads moved along the immobilized actin filaments and stochastically came off from the filaments after several seconds.

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Movie S1. Surface immobilization process of actin filaments. The movie focused on the bottom coverslip. The actin filaments sparsely biotinylated and fluorescently labeled had been anchored to the surface of the flow chamber through gelsolin. In the beginning of this movie, free ends of the filaments were fluctuating in the medium. After ~7 seconds, buffer flow containing streptavidin was reached to the filed of view. All the filaments were oriented to the downstream direction, and subsequently, were immobilized on the surface via biotin-avidin conjugation. Real time replay.