Supporting Information

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SI Text

Protein Preparation. Myosin II and actin were obtained from rabbit skeletal muscle and purified as described previously (1). Filamentous actin was stained with TRITC-phalloidin (Sigma-Aldrich) at a molecular ratio of $1:1,000$. For the myosin Va heavy chain construct, the human myosin Va heavy chain cDNA 3′ end (1–3714 bp) was deleted to obtain a myosin Va cDNA fragment that encoded amino acids 1–1,238. This fragment included the motor domain, neck domain, and coiled-coil domain. To ensure specific binding to the Q rod, two HaloTags were added downstream and in frame with the C terminus of the myosin Va via an Arg-Ile-Ala-Thr peptide. Flag-tag was linked to the N terminus of the myosin Va HC-HaloTag peptide for purification by affinity chromatography.

Motility Assay. Flow cells were constructed by sandwiching parafilm (50 μm thickness; Toray) with a quartz slide glass and cover glass (18 mm × 18 mm; Matsunami Glass).

Actin Sliding Assay. 10 μL of 0.5 mg/mL α-casein was introduced into the cell and incubated for 3 min. 5 mg/mL myosin II in motility buffer [MB; 25 mM KCl, 10 mM Hepes-KOH (pH 7.8), 5 mM $MgCl_2$, and 1 mM EDTA] was introduced into the cell, which was then incubated for 2 min and washed with 5 mg/mL α-casein in MB. 1 μg/mL sparsely labeled actin filaments in MB was flowed into the cell, incubated for 2 min and washed with 30 μL MB. Finally, MB with an oxygen scavenger (1), 5 μM ATP and an ATP-regeneration system was introduced into the cell. The specimen was sealed with enamel to prevent evaporation.

Myosin V Motility Assay. 10 μL of 1.5 mg/mL α-actinin was introduced into the cell and incubated for 3 min to allow for tight adsorption. Unbound α-actinin was removed by washing with 30 μL MB. 10 μL of 5 μg/mL actin was added and incubated for 3 min to allow for tight adsorption. Excess actin was removed by washing with 30 μL MB. The cell was then incubated with 10 μL of 5 mg/mL α-casein for 3 min to reduce particle adhesion. Excess α-casein was removed by washing with 30 μL MB. Finally, 5 nM Q rod conjugated myosin V in MB with an oxygen scavenger, 2 μM ATP, and an ATP-regeneration system was added into the flow cell, which was sealed with enamel.

Q Rod-Conjugated Myosin V Motility at 2 μM ATP. To see if the Q rod itself influences myosin motility, we observed Q rod-conjugated myosin V motility at 2 μM ATP (Fig. S4). The average step size of Q rod-conjugated myosin V was 36.5 ± 7.8 nm (mean ± SD). The histogram of Q rod-conjugated myosin V dwell times was fitted to a double exponential curve, yielding rate constants of $k_1 = 0.88 \pm 0.07\ \mu M^{-1}\,s^{-1}$ and $k_2 = 12.1 \pm 2.0\ s^{-1}$, which agree with the ATP-binding rate of $0.9\ \mu M^{-1}\,s^{-1}$ and ADP release rate of $12–16\ s^{-1}$ acquired from biochemical assays (2). The average translocation velocity was 58.9 ± 3.6 nm/s (mean <plus or minus > SE). These results confirm the Q rod did not affect myosin V motility.

Event Dwell Times. Dwell time histograms were fitted to the following double exponential curve.

$$\rho(t) = A\left(\frac{k_1 k_2}{k_1 - k_2}\right)(\exp(-k_1 t) - \exp(-k_2 t)),$$

where t is dwell time and A is a constant. Dwell times were determined by eye.

1. Harada Y, Sakurada K, Aoki T, Thomas DD, Yanagida T (1990) Mechanochemical coupling in actomyosin energy transduction studied by in vitro movement assay. *J Mol Biol* 216:49–68.

2. De La Cruz EM, Wells AL, Rosenfeld SS, Ostap EM, Sweeney HL (1999) The kinetic mechanism of myosin V. *Proc Natl Acad Sci USA* 96:13726–13731.

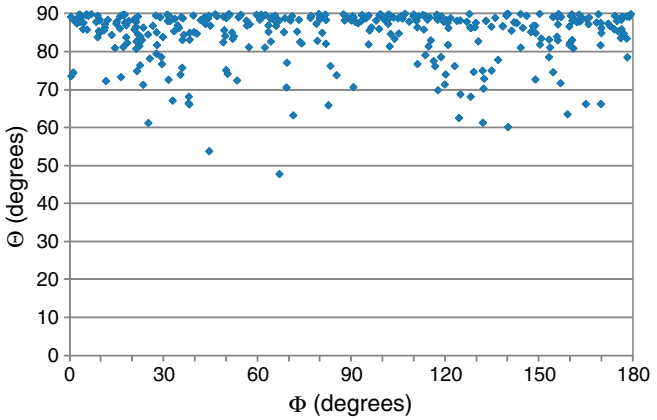

Fig. S1. Distribution of Q rods on a glass surface. Θ, the tilt angle, mostly converges at 90°, while Φ, the in-plane angle, distributes almost uniformly across all angles.

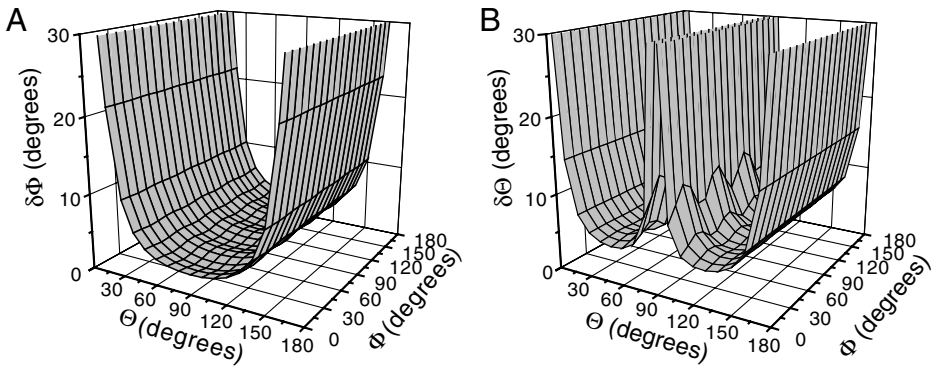

Fig. S2. The error surface of Φ (*A*) and Θ (*B*) as a function of dipole orientation (Φ, Θ).

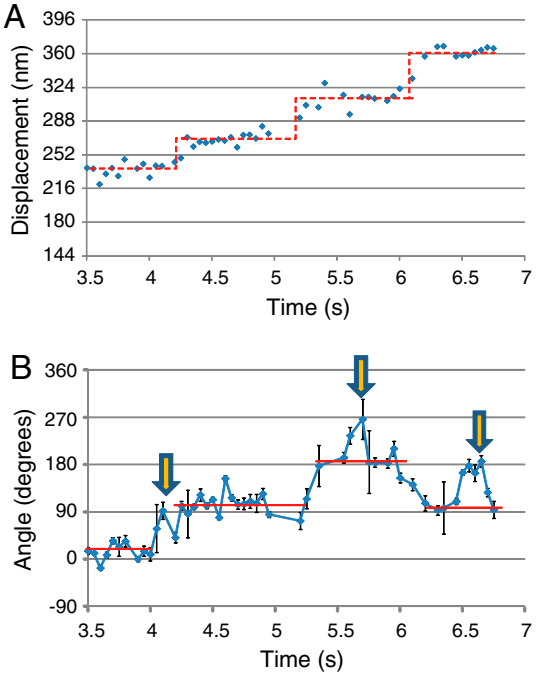

Fig. S3. Rotational motions not associated with normal steps. (*A*) Myosin V position. Blue circles, raw position data analyzed by FIONA; red dashed lines, the average position within a dwell period. (*B*) Changes in Φ. Blue circles, raw Φ values; red lines, dwell-averaged Φ. Vertical arrows indicate angle changes that do not coincide with steps. Error bars are SD.

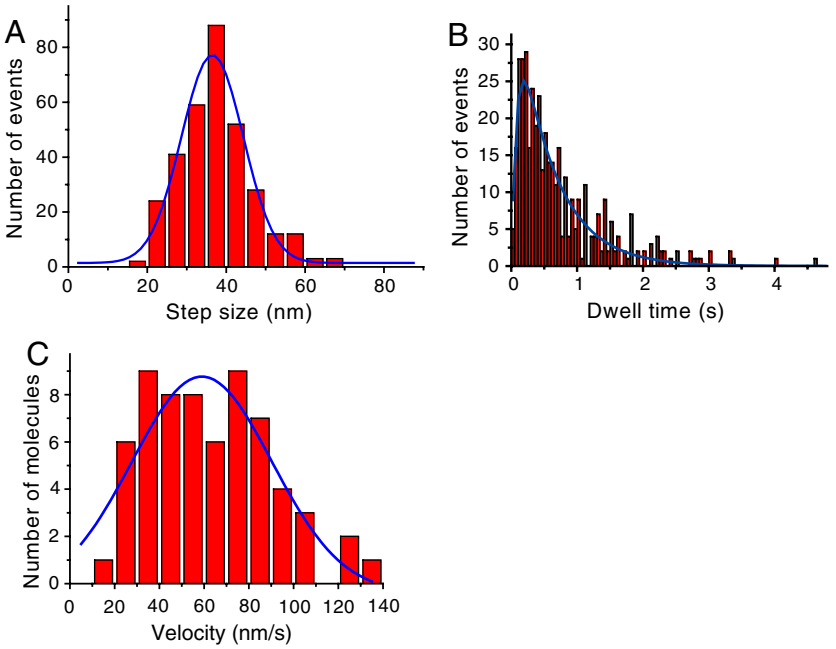

Fig. S4. Histograms of step size, dwell time, and translocation velocity. (*A*) Histogram of myosin V step sizes ($n = 320$). (*B*) Histogram of myosin V dwell times ($n = 441$). (*C*) Histogram of myosin V translocation velocities ($n = 64$). All observations were done at 2 μM ATP.

Movie S1. Axial rotation of an actin filament sliding over myosin molecules. The corresponding trace is shown in Fig. 3 *B* and *C*.

Movie S1 (AVI)

Movie S2. The rotation of myosin V about its own axis. The corresponding trace is shown in Fig. 4*C*.

Movie S2 (AVI)