Supporting Information

Local heat activation of single myosins based on optical trapping of gold nanoparticles

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Materials and Methods

Myosin construct

Human myosin V-HMM heavy chain construct (dimeric form myosin V, pFB1-flag-hMyosinVaHMM-HT-Myc): The 3'end (bp 3714 to 5484) of complementary human myosin Va DNA (Kazusa Product ID, ORK07567) was deleted to obtain a myosin Va-HMM cDNA fragment encoding a.a. Met1-Thr1238. This fragment includes the motor, neck, and coiled-coil domains. Flag-tag (two synthetic complementary oligonucleotides encoding a.a. Met-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-Arg) and HaloTag (Promega KK) genes were attached at the 5'end and 3'end of the myosin Va cDNA fragment, respectively, for Flag-tag selective purification and biotin labeling. The Myc-tag (two synthetic complementary oligonucleotides encoding a.a. Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) gene was attached at the 3'end of the HaloTag for detection. The Flag-hMyosinVaHMM-HT-Myc fragment was introduced into the Baculovirus expression vector pFastBac1 between the BamHI and HindIII sites.

Human Calmodulin construct: pFB1-hCaM. Calmodulin-coding cDNA (Kazusa Product ID, ORK11793) was also inserted into pFastBac1 between the BamHI and HindIII sites.

Human myosin V-S1 heavy chain construct (monomeric form myosin V, pFB1-HT-flag-hMyosinVa-S1-SNAP-His): human myosin Va cDNA (Kazusa Product ID ORK07567) was truncated at Gly924. This fragment included the motor domain, lever arm domain (IQ1-6) and a small part of the coiled-coil domain (11 a.a., SVERYKKLHIG). For biotin labeling, a HaloTag (Promega KK) was attached at the N-terminal via a linker (15 a.a., LRRRPTRPAMDPPSK). For oligonucleotide labeling and protein purification, SNAP-tag (New England Biolabs Inc) and 6 x

His-tag were attached at the C-terminal via linkers (2 a.a., RA). This Halo-hMyosinVa-SNAP-His fragment and the human calmodulin gene (Met1-Lys149, Kazusa Product ID ORK01403) were introduced downstream of the PH and p10 promoters of the Baculovirus expression vector pFastBac Dual, respectively.

Protein expression, purification and oligonucleotide labeling

Recombinant viruses for myosin V-HMM heavy chain and calmodulin were produced by homologous recombination using the Bac-to-Bac Baculovirus Expression System (Life Technologies). Sf9 insect cells were maintained in a monolayer culture in ventilated 175 cm^2 flasks at 28 ^oC with 10% FBS (Life Technologies) and 1×Antibiotic-Antimycotic liquid (Life Technologies)/EX-Cell 420 (SAFC Biosciences). After co-infection at a multiplicity of infection of 1 or 0.5, respectively, and incubation for 60 h, cells were collected by centrifugation at 1,500 g for 5 min at room temperature and stored at -80 ^oC. Frozen cells were suspended and sonicated in 10 ml per g cells of Lysis1 solution (30 mM Tris–HCl pH 8.0, 200 mM NaCl, 1 mM EGTA, 5 mM $MgCl_2$, 5 mM ATP, and 0.05 mM DTT) containing Complete, EDTA- free protease inhibitors cocktail tablet (Roche Diagnostics). After ultracentrifugation at 10,000 g for 20 min, soluble fractions were mixed with pre-activated Flag-affinity resin (ANTI-FLAG M2 Affinity Gel, Sigma-Aldrich Co. LLC.) for 1 h. After removal of the unbound protein, 1 mL Lysis solution and 2.5 μL HaloTag®-PEG-Biotin Ligand (Promega KK) were added at a molecular ratio of 1 : 6 to the resin, which was lightly incubated on ice for 10 min to achieve C-terminal biotinylation. Afterward, Wash1 solution (30 mM Tris–HCl pH 8.0, 200 mM NaCl, 0.1 mM EGTA, 5 mM $MgCl_2$, 2 mM ATP, and 0.05 mM DTT) was added to remove any unreacted

biotin ligand. Biotinylated protein was eluted by Elution1 solution (30 mM Tris–HCl pH 8.0, 200 mM NaCl, 0.1 mM EGTA, 5 mM $MgCl_2$, and 0.05 mM DTT) including 0.15 mM Flag peptide. The obtained myosin V recombinant (~0.3 mg ml^{-1}) was aliquoted and stored at - 80 ^oC until use. Biotinylation was confirmed by using a streptavidin-alkaline phosphatase conjugate and bromochloroindolyl phosphate/nitro blue tetrazolium as the substrate. All steps were performed at less than 4 ^oC unless stated otherwise.

Monomeric form myosin V (S1) was purified as follows. Recombinant viruses for myosin V heavy chain and calmodulin were produced by homologous recombination using the Bac-to-Bac Baculovirus Expression System (Life Technologies). Sf9 insect cells were maintained in a monolayer culture in ventilated 175 cm^2 flasks at 28 ^oC with 10% FBS and 1×Antibiotic-Antimycotic liquid/Sf-900 II SFM (Life Technologies). After 60 hours of incubation for recombinant proteins expression, cells were collected by centrifugation at 6,000 g for 5 min and stored at - 80 ^oC. Frozen cells were suspended and sonicated in 2 ml Lysis2 solution (30 mM Tris–HCl pH 8.0, 200 mM NaCl, 1 mM EGTA, 5 mM $MgCl_2$, 5 mM ATP, and 10 mM 2-mercaptoethanol) containing Complete, EDTA- free protease inhibitors cocktail tablet (Roche Diagnostics) per 175 cm^2 flask of cells. After ultra centrifugation at 35,000 g for 20 min, soluble fractions were mixed with pre-washed 100 μL Ni-NTA Agarose (QIAGEN) for 40 min. Afterward, Wash2 solution (20 mM Tris–HCl pH 8.0, 300 mM NaCl, 0.2 mM EGTA, 1 mM $MgCl_2$, 1 mM ATP, 10 mM 2-mercaptoethanol and 20mM imidazole pH8.0) was added into the column to remove unbound proteins. Myosin was eluted by Elution2 solution (20 mM Tris–HCl pH 8.0, 100 mM NaCl, 0.2 mM EGTA, 1 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 300 mM imidazole pH8.0), and myosin V recombinants were obtained (~0.3 mg ml^{-1}).

For S1, labeling reactions of oligo A and B were performed just after His-tag affinity purification. Amine modified DNA oligonucleotides (oligo A and B, Hokkaido System Science) were linked to the SNAP substrate, benzylguanine (BG) (New England Biolabs), and 25 μM of BG-oligonuculeotides was labeled with ~ 1 μM S1 containing an C-terminal $SNAP_f$ tag (New England Biolabs) in His tag affinity Elution3 buffer (20 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM $MgCl_2$, 0.2 mM EGTA, 300 mM Imidazole pH8.0 and 10 mM 2-mercaptoethanol) for 30 min at room temperature. Oligo-labeled myosin V was purified by an actin filament affinity, aliquoted and stored at -80 ^oC until use. Just before the experiment, ~ 500 nM oligo-A myosin V and oligo-B myosin V was mixed together and incubated for 30 min at 4 ^oC to form DNA dimerized myosin V dimers. Labeling efficiency of oligonucleotides with myosin V and forming of dimerization was estimated by a gel-shift assay (4-15 % gel, Biorad) and confirmed ~ 100 % efficiency of labeling for both oligo A and B. Myosin V was visualized with CBB stain (Nakalai tesque).

Dark-field microscopy combined with optical tweezers

Dark-field microscopy was modified from previously reported methods[1, 2] in order to incorporate optical tweezers (**Supplementary Figure 1**). Illumination of the dyes and GNPs was achieved by 532 nm laser light (Photop Technologies, DPGL2100-F). An incident laser beam was steered to the outer edge of the back aperture of the objective lens (Olympus, ×60, N.A. = 1.45, oil) to produce near evanescent illumination. Fluorescence from cy3-GNPs, actin filaments and scattered light from GNPs were passed through dichroic mirror 1, a perforated mirror, tube lens and relay lens. Scattered light was divided from the fluorescence by dichroic mirror 2 and projected onto a quadrant photodiode (Sentech, OP711A, response time ~ 20 μs)

through a spherical lens to magnify the image 1000-fold and then a beam sampler (Thorlabs, BP108). 8% of the scattered light was projected onto a CCD camera (Thorlabs, DC210), and the dark field image of the optically-trapped GNP was detected (**Supplementary Figure 1B**). Fluorescence images were detected by an EMCCD camera (Andor, DL658M-NIT) at a 30 Hz recording rate. For optical trapping, 1064 nm laser light (Spectra Physics, J20-BLS-4W) was collimated, expanded and passed through an electro-optic deflector (Conoptics, 302RM) for the steering of the beam. The laser intensity was adjusted by a motorized half wave plate and polarized beam splitter.

Construction of DNA rod

DNA rods to link GNPs and myosin V were constructed based on a previous report[3]. A nanosized rod structure was made using bundles of double stranded DNAs (DNA origami). To construct the nanostructure, >7000 base single stranded DNA ("scaffold") and >100 species of oligo nucleotides ("staples") were required. In our work, we used M13mp18 phase DNA (New England Biolabs) as the scaffold, and 151 species of staples (Hokkaido system science) (**Table S1**) were prepared. To link one end of the DNA rod with avidin GNPs, three 3' biotin oligo nucleotides were prepared (**Table S2**). We added handle oligo nucleotides (**Table S2**) to link myosin V 87 nm (257 bp) and 166 nm (489 bp) from the biotin oligo nucleotides. The folding reaction to produce DNA origami was carried out in Folding buffer (10 mM Tris [pH 8.0], 1 mM EDTA and 22 mM $MgCl_2$) by rapid heating to 80 ^{o}C and then cooling in single degree increments to 60 ^{o}C over 120 min, followed by additional cooling in single degree increments to 25 ^{o}C over 72 hr.

Folded DNA origami was purified by agarose gel electrophoresis (2%, 50 V, 3 hr in TBE buffer (25 mM Tris, 24 mM boric acid, 1 mM EDTA) with 11 mM $MgCl_2$). Well migrated single bands (**Supplementary Figure 3A**) were excised, crushed and spun through a Freeze and Squeeze column (Bio-Rad) for 10 min at 13,000 g and 4 ^{o}C. DNA rods were stored at 4 ^{o}C.

Preparation of gold nanoparticles and labeling with myosin V

Preparation of Cy3-streptavidin labeled GNPs was done as described[1] with some modifications. Briefly, 18 pM colloidal gold (60 or 80 nm in diameter, British BioCell International) was mixed in alkane thiol solution (1 mM PEG6-OH alkanethiol, 0.1 mM biotynilated PEG alkanethiol (SensoPath Technologies)) and incubated 24 hr at 70 ^{o}C to generate self-assembled monolayers on the gold surface. Unbound alkane thiol was removed by centrifugation at 500 g for 15 min three times. Then, biotinylated PEG-coated gold was mixed with avidin solution (0.1 mg/ml cy3-streptavidin (Life technologies), 0.5 mg/ml neutravidin (Life technologies), 5 mg/ml BSA(Sigma-Aldrich), 0.02% tween 20 (Wako)) and stocked at 4 ^{o}C. The stocked gold was centrifuged at 500 g for 15 min to remove unbound avidin molecules. The pellet was suspended in 10 mg/ml BSA in 4 mM HEPES-KOH [pH 7.8] to adjust the GNP concentration to 180 pM just before use.

Cy3-streptavidin labeled gold nanoparticles were mixed with biotinylated myosin V at a molar ratio of 1 : 10 and incubated for 3 hr. At this condition, less than 20% of gold showed interactions with actin filaments, which strongly suggests interaction events were caused by single myosin V molecules[4]. For the preparation of complexes that included a gold nanoparticle, myosin V dimer and DNA rod, ~ 500 pM of DNA rod was mixed with 150 pM avidin-coated GNP and 100 nM myosin V dimer and incubated overnight.

Single molecule optical trapping assay

Preparation of the sample chamber and optical trapping assay was performed as previously described[2,4]. Briefly, fluorescently labeled actin filaments were quickly flowed into a sample chamber in which α-actinin was directly adhered to a glass slide. The glass surface was then coated with 5 mg ml^{-1} casein. Myosin V tagged with 80 nm gold in Assay buffer (20 mM HEPES-KOH [pH7.8], 25 mM KCl, 5 mM $MgCl_2$ and 1 mM EGTA) containing 0.05 % tween, 1 mM ATP and an oxygen scavenger system (0.11 mg ml^{-1} glucose oxidase, 18 μg ml^{-1} catalase, 2.3 mg ml^{-1} glucose and 0.5 % 2-mercaptoethanol) was introduced into the flow chamber and sealed with nail polish.

Gold nanoparticles were visualized by Cy3 fluorescence on optically-trapped GNP and projected onto a fluorescent actin filament attached to the glass surface. The intensity of a 532 nm laser was used to fully visualize the dark-field image of the optically-trapped GNP, and the motion was detected by a quadrant photodiode. Single-molecule assays were performed at 25 ± 1 ^{o}C.

Data analysis

Data analysis was done as described previously[5]. Briefly, the displacement of an optically-trapped particle was recorded at a sampling rate of 24 kHz and bandwidth of 10 kHz. The trace was passed through a low pass digital filter (Chebyshev type filter by commercial software, DaDiSP 6.5, CAE Solutions) to reduce Brownian noise, and the step size and dwell time were estimated from a step-finding algorithm[6] or by eye. The load exerted on the particles was calculated from the displacement multiplied by the trap stiffness (6-10 fN/nm), which was

determined from the variance of the Brownian motion of a trapped particle by the equipartition theorem of energy. Dwell times shorter than 10 ms were excluded from analysis due to the uncertainty of the temporal resolution.

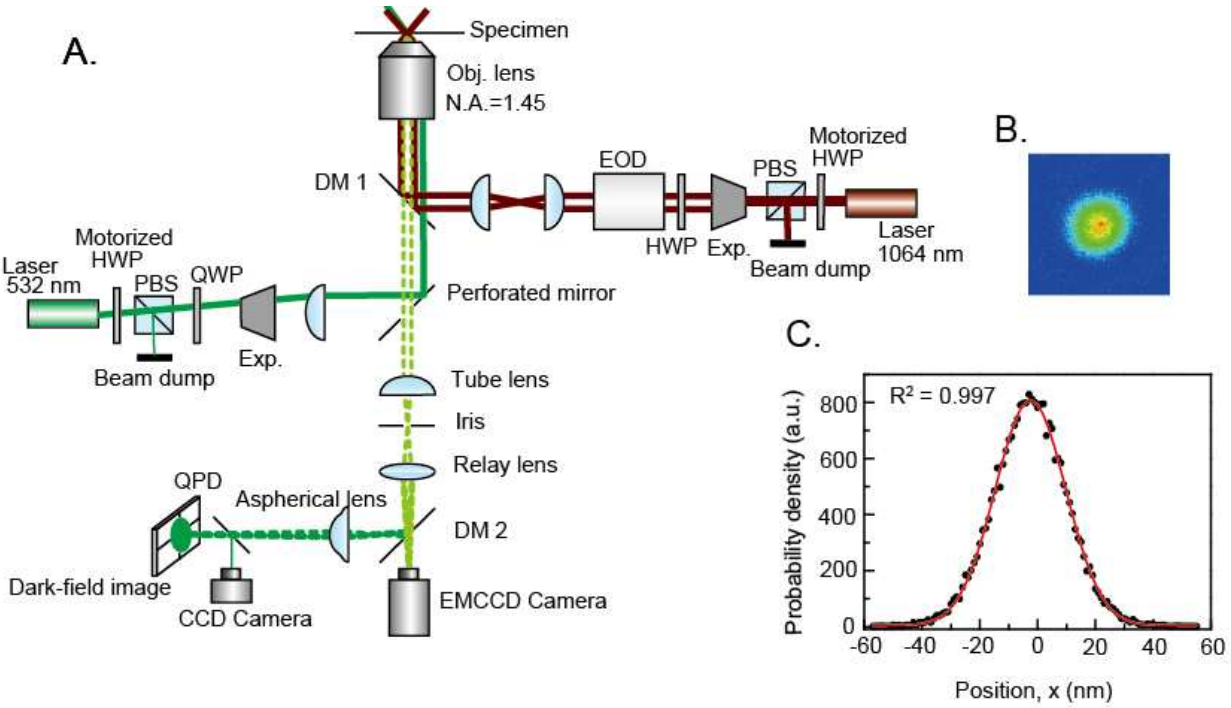

Figure S1. Optical setup and demonstration of GNP (ϕ = 60 nm) trapping and detection.

(A) Schematic drawing of objective-type dark-field microscopy with optical trapping. A 532 nm laser was used for both fluorescent and dark-field image observation. An EMCCD camera detected fluorescent images of GNP and actin filaments to interact with each other. When GNP with myosin V was positioned onto the actin filaments, 532 nm laser intensity was increased and the dark-field image of GNP was monitored by a quadrant photo diode (QPD) with high spatio-temporal resolution. An electrooptic deflector (EOD) was used for stable and rapid steering of the trapping laser beam (1064 nm). This setup enabled us to detect optically-trapped small particles with a high S/N ratio and manipulation on a μs time scale. DM, dichroic mirror; PBS, polarized beam splitter; HWP, half wave plate; QWP, quarter wave plate; Exp., expander. **(B)** Dark-field image of an optically trapped gold (ϕ = 60 nm) detected by a CCD camera just before

the QPD. The CCD camera (15 Hz flame rate) was used to confirm the shape of the dark-field image of optically-trapped GNP to ensure linearity between GNP displacement and the QPD output signal. (C) Probability density of the one-dimensional position of the trapped GNP has a Gaussian distribution, suggesting the trap potential obeys Hooke's law.

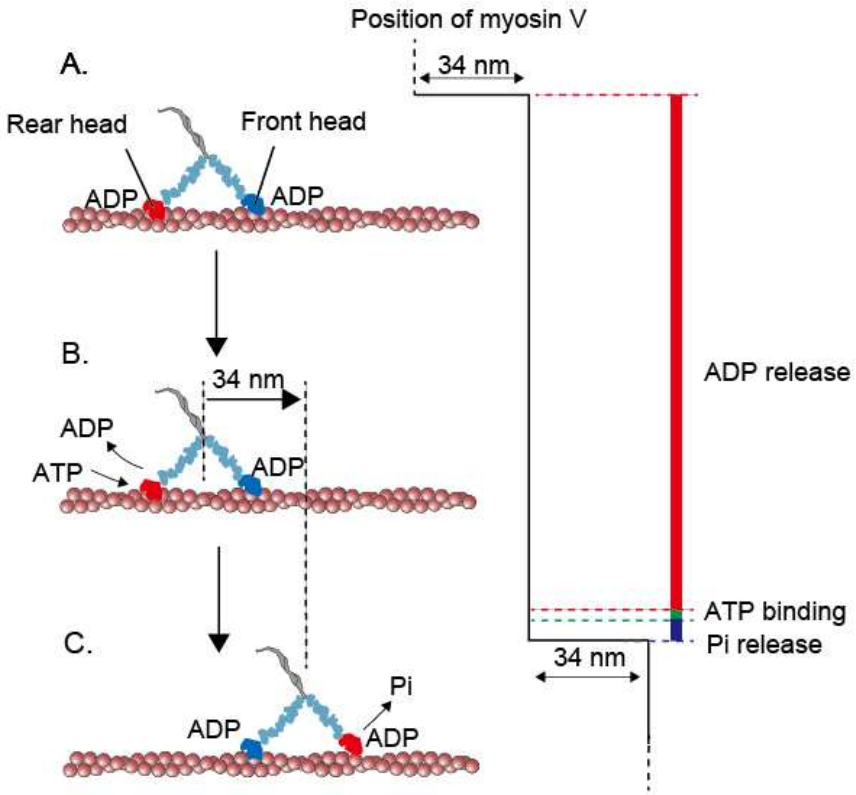

Figure S2. Tight coupling between ATP hydrolysis and myosin V steps.

(A) Both heads strongly bind to an actin filament during the ADP bound state. **(B)** ADP release from the rear head is the rate-limiting step. After its release, ATP binds to the head. In our experiment, because ATP concentration is high, ATP binding occurs rapidly. **(C)** After ATP binding, the rear head detaches from actin and a 34 nm step occurs. These series of events happen for each step, i.e. tight coupling.

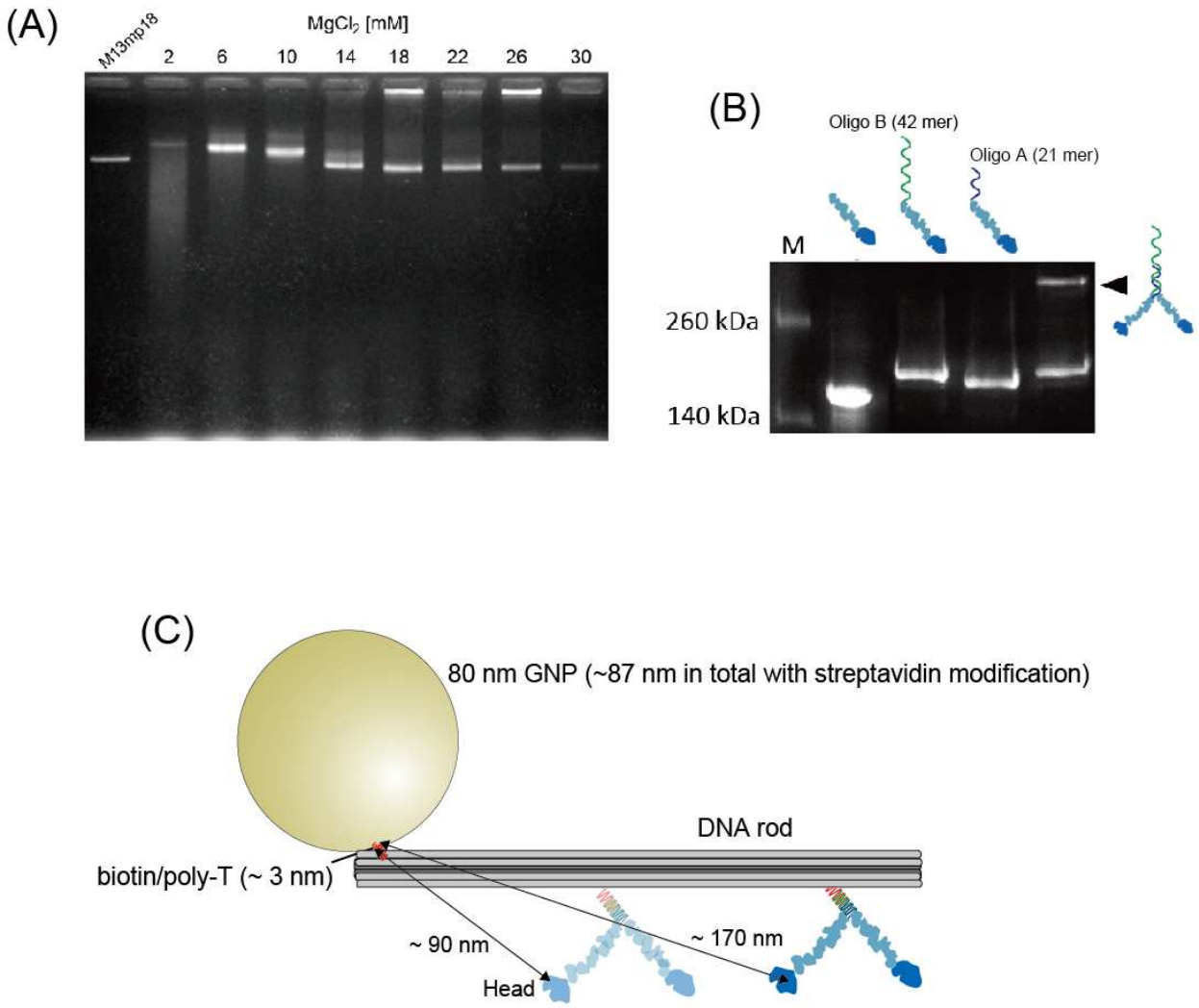

Figure S3. Gel analysis of a twelve-helix bundle DNA rod and geometry of the DNA origami complex (see main text for definition).

(A) DNA rods folded at different $MgCl_2$ concentrations. Samples at > 22 mM $MgCl_2$ migrated well, suggesting sufficient folding of the DNA rod[7]. **(B)** SDS-PAGE showing dimerization of myosin V monomers through DNA hybridization. M, Marker. Arrowhead indicates dimer population. **(C)** Distance between the GNP surface and motor domain of myosin V.

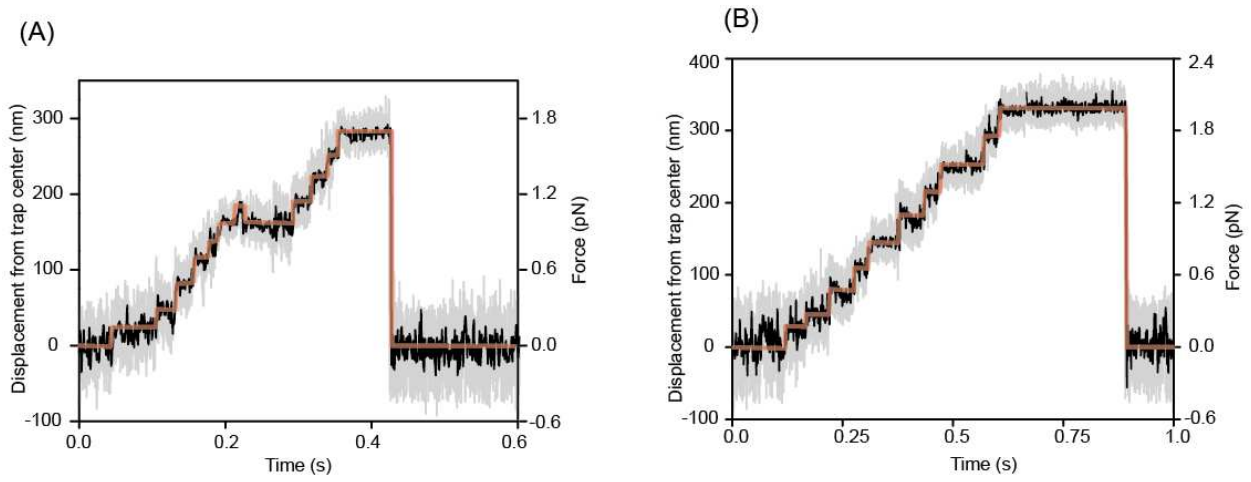

Figure S4. Typical trajectory of processive steps of GNP-DNA rod-myosin V complexes (DNA origami complexes).

(A) Trajectory of a complex with ~ 90 nm separation between the GNP surface and myosin head. Gray line: raw trace at 24 kHz sampling rate; black line: low pass filtered trace at 500 Hz. **(B)** Trajectory of a complex with ~ 170 nm separation between the GNP surface and myosin head. Gray line: raw trace at 24 kHz sampling rate; black line: low pass filtered trace at 200 Hz; red line: fitted trace using an automated step finding algorithm[6]. Trap stiffness: 6 fN/nm; ATP concentration: 2 mM; room temperature: 25 ± 1 ^{o}C.

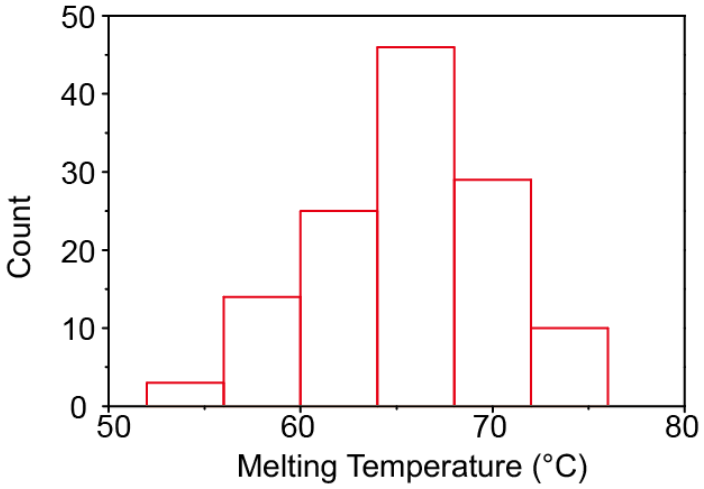

Figure S5. Melting temperature of staples in DNA rods

Staples of DNA rods included 151 species of 25-54 mer oligo nucleotides and showed a melting temperature of 65.5 ± 4.5° C (Mean ± S.D.). See **Tables S1 and S2** for a description of the staples.

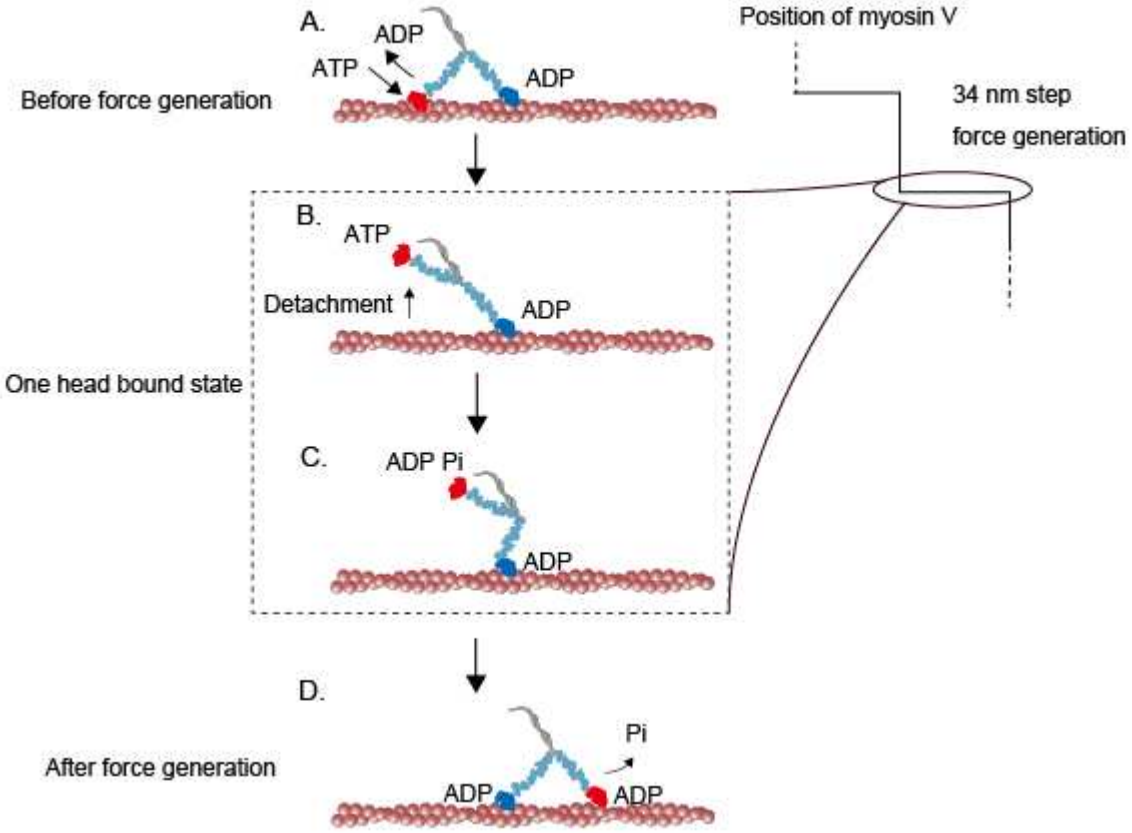

Figure S6. Force generation.

(A) ATP binds to the rear head. **(B)** ATP binding induces detachment of the rear head from actin. **(C)** The front lever arm of the rear head tilts forward and the head undergoes Brownian motion. During states B and C, myosin V remains attached to the actin filament via the front head. If the front head detaches from actin, force generation fails and myosin V diffuses away. **(D)** The rear head reattaches at a forward actin target, completing the force generation and 34 nm forward step.

Table S1. Core staples used to build twelve double-helix bundles

CGGCTTAGGTTGGGTTATCGAATTA
GAAAAACCACGCTGGTTGCCAACGGTGTTTT
TCAATCCTTTTTTAATGGAATACCAAAATCTA
CGTTATTAATTTTAAAAGTTACAGTACATAAA
AGTCTGTAGCCAGCTTTCCGTACGACGGTTAA
GTTCCAGATAGCCCGGGCGCGTACTCGCTTAATG
CACGACGATTCGCCGGAGCTGTTAACCAGGAGCTT
GCCAGGTCAAATTTTTGTTAATCAAAAATAATTCG
GCCCAATTCAAATTAAGCAATACCAAAAACATTAT
GTATAGCGATGACTATTATAGAAGAGGAAGCCCGA
TCACTGACCAGTAAATTGGGCTGAATTACCTTATG
CGCAGCTTGGGGTAGCAACGGATGAGGAAGTTTCC
CTGGTTTTGAGGAACCCATGTCTACAACGCCTGTA
GCTCATAGCAGGTTGAGGCAGATAAATCCTCATTA
ACAGAAGGAAAATTCATATGGCAACCGATTGAGGG
GAGTATTCTATTATTTATCCCTCAAAAATGAAAAT
AGTACTAGATAAAGTAATTCTCTAATGCAGAACGC
AAATTCAGAGATAGATGAGTTTGAGATCAGGCTGC
TCAACTACGACTCTAGCTTCGCTATTACGCCAGAG
CTTTAAACATTTTTTATCAATCATATGTACCCCCG
AGCTTTACAGAGCAAATTAGCTATATTTTCATTAT
GTCAGTACAAAGCGAGGCGTCCAATACTGCGGATT
TACGAGAGGTTTAACTATCAAGAGTAATCTTGAAA
CGTAAACGGCTTTGTACCATCGCCCACGCATAAGG
ATTTATTCACTGAGGCTGTATCACCGTACTCAGGC
TCATTGTGATTGGCTAGGAACCGCCTCCCTCAGTG
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GAACAAAATAAGAATCAAGATTAGTTGCTATTTCA
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TATAATCAGTGAGGGCATTAAAGTCGGGCTGTGTG
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CTGAATGCTAGCTTAATAATTTCATTTGAATTACCC
CAAAATCATAAACAACATGTTCAGGTCCAGAATCAATA
CGCCGCTAGTTATCCGCTCACAATTCCACACAACTCTGGTG
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AACGACTGATAGTTGCGCCGACAATGACAACAAAGTGCCGTC
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CTTTCCATAGGATTAGCGGCAATAATAAAAAGGAACAACTAA
ACATGGCCGGCATTTTCGGAGGGAACCTACAGTTAATGCCCC
CACCGACAACAAAGTTACCGGCGTAATCCCAATGAAACCATC
AGAATTAAAGGCTTATCCGATAATGAAAAAGCCCAATAATAA
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AAGAGAGATAACCCACAAGAATTGAGTTTACAGATATATTGAGCGCTAA
CTTCCAAGAACGGGTATTAAACCAAGTAATAATAAACCTGTCTTTCCTT
TTCGCGAGAAAACTTTTTCAAATATATTTGGCGCAGAAATCGCAAGACA
CTGAGAACGCGGGGTGATGGTTGCTTTGACGAGCCTGAATCGTGCCAGT
AATATTTAAAATGTGCTGCAAGGCGACCCTGGCGAAAGGGGCACTCCCCATCAC
GCATTAACATAAAGCCCCAAAAACAGTTGGTTGATAATCACGGAGAGGTAATAA
TCAGAAAACGAAAAGGTGGCATCAATGATGGGGCGCGAGCTACGGTGGCCTTGC
ATTCAGTGAATCATTGAATCCCCCTCTCATCGTCATAAATTATCATAGCAACAG
TTGCGGGATCTCATTACCCAAATCAAAGCAAGAACCGGATGCCGGAATCAATCG
ACCCTCAGAGCGCTGAGGCTTGCAGGTCCCGATATATTCGAAAAAGGGTCACAC
GCCGCCACCACCACCCTCAGAACCGCAAGAGGTTTAGTACAGAGGCTATAGAAC
CGCAAAGACAGAACCGCCACCCTCAGATAGCCGCCACCCTTTAGCGTGACAATA

CGTCTTTCCAACATACATAAAGGTGGATCAAACGTAGAAACTTTTTAGATAGCC
CATTTTCGAGATTTTATCCTGAATCTAATGCACCCAGCTACGCCCAACCAGCAG
ACCTTGCTTCTTAACAACGCCAACATGAGAGAATCGCCATAAATAAGCCGCCTG

Table S2. Handle staples to link GNP and myosin V.

Oligo A	NH_2-TTCACTACTTACCACTCTACC
Oligo B	TGGATATGGTGGAGAGGAGAGGGTAGAGTGGTAAGTAG TGAA- NH_2
Handle for 87 nm separation	TTCACCACTCCAAAAGGAGCGGACAGCAGAGTTAAAGG CCGCTT*CTCTCCTCTCCACCATATCCA*
Handle for 166 nm separation	GTTGGGTAACGCCAGGGTTTTCCCCCAGCAGGC*CTCTCCT CTCCACCATATCCA*
Biotin handle 1 to link with GNP	CTTTCAATTATCCTTGAAAACAGTCAATAGTGAATTTAT*T TTTTTTTTT*-biotin
Biotin handle 2 to link with GNP	GGGTAAGACTGAACACCCTGAACAGAAAAATAATA*TTTT TTTTTT*-biotin
Biotin handle 3 to link with GNP	ATCGGACACGAGCATGTAGAAACTTAACCTC*TTTTTTTTTT* -biotin
Biotin handle 4 to link with GNP	TTCATGAAATTTAATTAGTTAATTTCAT*TTTTTTTTTT*-biotin

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