nature nanotechnology

SUPPLEMENTARY INFORMATION

DOI: 10.1038/NNANO.2015.132

Mechanical coordination in motor ensembles revealed using engineered artificial myosin filaments

R. F. Hariadi[‡], R. F. Sommese[‡], A. S. Adhikari, R. E. Taylor, S. Sutton, J. A. Spudich, and S. Sivaramakrishnan[*]

[‡] R. F. H. and R. F. S contributed equally to this work.
[*] Correspondence to: E-mail: sivaraj@umn.edu

Supplementary Materials

Supplemental Materials and Methods
Figures S1 to S4
Tables S1 to S2
Movies S1 to S3

Supplemental Materials and Methods

Agarose Gel Assay.

To quantify nanotube labelling, Cy5-labelled nanotubes were mixed with Cy3-*a'* or Cy3-*b'* in TAE/Mg^{2+} buffer + 1 mg/mL BSA and incubated for one hour at room temperature. Samples were run on a 0.8% agarose gel in TAE/Mg^{2+} buffer for 90 minutes at 60 V. The gel was imaged on a Typhoon gel imager (GE Healthcare) and quantified using ImageJ (Supplementary Fig. 4).

Preparation of Benzyl-Guanine Oligo.

Benzyl-guanine NHS ester (BG-GLA-NHS; NEB) was covalently linked to C6-amine Cy3-modified oligonucleotides (C6-amine-Cy3-*a'*). Briefly, 0.17 mM C6-amine-Cy3-*a'* was incubated with 11.6 mM BG-GLA-NHS in 0.1 M $NaBO_3$, pH 8.5 for ~4 hours at with shaking. The BG-labelled Cy3-*a'* DNA labelling solution was then purified twice through Illustra G-50 micro columns (GE Healthcare) equilibrated in 2 mM Tris, pH 8.5. The final BG-labelled Cy3-*a'* (BG- Cy3-*a'*) concentration was determined by measuring Cy3 absorbance.

Standard Gliding Assay.

For standard gliding assays with β-cardiac myosin, a PDZ-based affinity tag system was used as previously described[1]. Briefly, PDZ-clamp was incubated in flow chambers at 5-20 uM for 2–4 minutes. Excess PDZ-clamp was washed out, and the surface was incubated with AB.BSA (AB + 1mg/mL BSA; AB: 25 mM Imidazole, pH 7.4, 25 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT) for 2–4 minutes. Finally human β cardiac myosin was incubated on the surface for 2-4 minutes, washed out with AB.BSA, and the final actin imaging solution was added containing Alexa-488 phalloidin-labelled (Invitrogen) F-actin, 1 mM ATP, 1 mM phosphocreatine, 0.1 mg/mL creatine-phosphokinase, 45 μg/mL catalase, 25 μg/mL glucose oxidase, and 1% glucose. Motility was tested with and without dead-heading, and standard gliding assays speeds compared to previously measured speeds for this myosin construct[2].

Nanotube Imaging.

Nanotubes were imaged at 150x magnification on a Nikon TiE microscope equipped with a 100x 1.4 NA Plan-Apo oil-immersion objective, 1.5x magnifier, mercury arc lamp, Evolve EMCCD camera (512 pixel x 512 pixel; Photometrics), Nikon Perfect Focus System, and Nikon NIS-Elements software. To create flow chambers, channels ~3 mm apart were made with parafilm on a glass slide. The glass slide was heated and coverslips coated with 0.1% collodion in amyl acetate (EMS) were adhered. Nanotubes were then immobilised to the coverslip surface at room temperature as follows. Biotinylated-BSA at 0.1 mg/mL in AB was incubated for 2–4 minutes. Excess biotin-BSA was washed out, and the surface was incubated with AB.BSA.casein (AB + 1 mg/mL BSA + 1 mg/mL casein) for 2 minutes. Next, neutravidin at 0.1 mg/mL in AB.BSA was incubated for 2–4 minutes. Excess neutravidin was washed out with AB.BSA. Finally nanotubes were added at 2–5 nM concentration in AB.BSA.DNA (AB.BSA + 5–10 nM random DNA mix) and incubated for 3–5 minutes. Excess nanotubes were washed out of the chamber with AB.BSA.DNA.

For myosin labelling, myosin V or VI was diluted to ~0.1 μM in AB.BSA.DNA with 8 μM calmodulin added. In the case of myosin V, the solution was pre-cleared of any myosin V multimers and aggregates through a high speed spin ~220,000x g for 15 min at 4 °C. For β-

cardiac myosin, motor was used immediately after preparing and diluted to ~0.1 μM in AB.BSA.DNA. The nanotube-coated chamber surface was incubated with 3x10 minute washes of ~0.1 μM myosin. Finally, the chambers were carefully washed of excess myosin through multiple washes of AB.BSA or AB.BSA supplemented with ~8 μM calmodulin. The final wash contained 25 μg/mL glucose oxidase, 45 μg/mL catalase and 1% glucose (wt/v). Nanotubes were imaged and the Cy3 and Cy5 intensities were quantified using custom Mathematica algorithms. Briefly, individual nanotubes were manually selected using ImageJ. Images were skeletonised and regions were nanotubes crossed one another were excluded from further labeling efficiency analysis. In the Mathematica algorithm, the Cy3 intensity was normalised by the corresponding Cy5 intensity for each detected pixel.

Trajectory Analysis.

Actin trajectories were analysed using either custom Matlab nanotube-tracking code[3] or Imaris (BitPlane). In the MATLAB software, frames were thresholded into binary images, and filaments were found and labelled using the MATLAB's Connected Components function (bwconncomp). Moving filaments were tracked between consecutive frames based on the areas of the filaments and proximity of their centre of masses. In both cases, with myosin V and VI, analysis of gliding actin filament was restricted to filaments that were longer than 3 pixels (320 nm), appeared for more than 8 seconds, and travelled for more than 3 pixels (320 nm). For β-cardiac myosin, due to the lower number of events, movies were analysed to identify actin-nanotube gliding events with filaments that move along DNA nanotubes for at least 4 frames (~1 sec). The filament lengths were measured by determining the major axis of each filament. To exclude false positives, actin filaments that temporarily slowed down (frame-to-frame speed <25 nm/sec) were excluded from further analysis. Gliding speeds were measured by dividing the distance between consecutive centroids with the exposure time (0.25–1 sec).

Stochastic Simulation of the Actin Gliding Movement.

A custom Mathematica code was used to simulate the sliding movement of an actin filament with continuous binding sites of length L over a periodic one-dimensional myosin chain of ensemble size $N=L/\lambda$, where λ is the myosin spacing (Fig. 3; Supplementary Movie 2). Individual myosin comprises two heads. In our model, because of the high duty ratio of myosin V and myosin VI[4], one of the two heads is assumed to be always bound to an actin filament and consequently, the duty ratio was set to 1. Each myosin is referred to by an integer index (i=1–N). Myosins are attached to a rigid linear backbone (grey) by a mechanical linkage with spring constant k.

The simulation of sliding of the actin filament is initiated with a zero strain configuration or $\Delta x_i = 0$, where Δx_i is the local displacement of myosin i from the zero strain position. The power stroke of each myosin motor is stochastic with a single exponential dwell time distribution having a mean of 1/(single myosin speed). In Fig 3c–e, the single molecule myosin V and VI speeds at 2 mM ATP were set to be 378 nm and 146 nm/sec, respectively[5]. Initially, N independent exponentially-distributed dwell time sequences for N myosin motors were generated. The reaction time was calculated by consecutively adding the dwell times.

The following sequence was repeated until a maximum number of kinetic steps were reached:

(1) In our model, a power stroke will occur for a motor with: (i) the earliest reaction time and (ii) a resulting tension $T = k.\Delta x$ less than stall force (F_{stall}). A successful power stroke produces a forward step of 36 nm[6]. Because of the high-duty ratio of myosin V and VI[4], the release, stroke, and rebinding reactions of myosin were considered instantaneous.

(2) Mechanical equilibration along the actin filament (amongst all myosin) occurs instantaneously after a power stroke. This condition is reached by adjustment of all position of myosin heads by s/N, where s is the step size of the myosin (36 nm)[6]. This displacement will alter the local tension of myosin i, to $T_i = k.\Delta x_i$.

(3) Upon completion of mechanical equilibration, the reaction time is updated based on the rate of the local power stroke. The power stroke rate of myosin i depends on its updated tension T_i. The force-dependent reaction rate was calculated based on the piece-wise function below, where v_{max} is the speed of single myosin motor on an actin filament[5].

$$v(T) = \begin{cases} v_{max} & , \text{if } T \leq 0, \\ v_{max}\left(1 - \frac{T}{F_{stall}}\right) & , \text{if } 0 < T < F_{stall}, \\ 0 & , \text{if } T \geq F_{stall}, \end{cases}$$

Supplementary References

1 Huang, J., Nagy, S.S., Koide, A., Rock, R.S. & Koide, S. A peptide tag system for facile purification and single-molecule immobilization. *Biochemistry* **48**, 11834-11836 (2009).

2 Sommese, R.F. *et al.* Molecular consequences of the R453C hypertrophic cardiomyopathy mutation on human β-cardiac myosin motor function. *Proc. Nat. Acad. Sciences* **110**, 12607-12612 (2013).

3 Churchman, L.S., Okten, Z., Rock, R.S., Dawson, J.F. & Spudich, J.A. Single molecule high-resolution colocalization of Cy3 and Cy5 attached to macromolecules measures intramolecular distances through time. *Proc. Nat. Acad. Sciences* **102**, 1419-1423 (2005).

4 De La Cruz, E.M. & Ostap, E.M. Relating biochemistry and function in the myosin superfamily. *Curr. Opin. Cell Biol.* **16**, 61-67 (2004).

5 Ali, M.Y. *et al.* Myosin Va and myosin VI coordinate their steps while engaged in an in vitro tug of war during cargo transport. *Proc. Nat. Acad. Sciences* **108**, E535-E541 (2011).

6 Rock, R.S. *et al.* Myosin VI is a processive motor with a large step size. *Proc. Nat. Acad. Sciences* **98** (2001).

Supplementary Tables and Figures

Supplementary Table 1
Staple strand sequences for the 10-helix nanotubes (Supplementary Fig. 1).

Core Single-Stranded Tile (SST)	
UM-01	5'-CGCAGTACAGTCCGTGACTAACCATCCGTTTAGGCATCCTTG-3'
UM-02	5'-TAAACGGATGGCCTTATCGGCGTGCCAGAGAGTCGCTAGTTA-3'
UM-03	5'-GTGCTTCCACTAACTAGCGACGATCAGGGTCTCAGAACGCTT-3'
UM-04	5'-AGACCCTGATCCGTCACAAGCAAGAGCGAGGTTGAGAACACA-3'
UM-05	5'-CCATACCGTCTGTGTTCTCAAATCATGTGCAATTCATCACCG-3'
UM-06	5'-TTGCACATGATCCGAATCCTGCCACATTGCTGGTTGATGTTG-3'
UM-07	5'-TTCGCCATGTCAACATCAACCCAAGCCTACAATTCGCAAGAC-3'
UM-08	5'-TTGTAGGCTTGTTCGGTTCGATGGTAGAGGCTGACTTGATGG-3'
UM-09	5'-TGGTCATCGTCCATCAAGTCAAGATTCTCGTCAATGGTCCCC-3'
UM-10	5'-GACGAGAATCTGTTTCCCGACACGATGTGTCTTAGTCACGGA-3'
UM-11	5'-GACACATCGTAAGTCGTCTTCAGAGTTGTTCAGCCGATAAGG-3'
UM-12	5'-TGAACAACTCTACGCAGTGATCTTTGCCCTCTAGGTGCTCTA-3'
UM-13	5'-TCTCTGGCACTAGAGCACCTATTGGCTTAGACGCTTGTGACG-3'
UM-14	5'-GTCTAAGCCAAGTCCACTCGTAAGGGACACCTGCCACTATTG-3'
UM-15	5'-CCTCGCTCTTCAATAGTGGCATAACAGACCTCCAGGATTCGG-3'
UM-16	5'-GAGGTCTGTTAGCCTAGATCGCGGAATACGGTGTCATACACC-3'
UM-17	5'-AGCAATGTGGGGTGTATGACAGTATATGCAGCTCGAACCGAA-3'
UM-18	5'-GCTGCATATACATCTCCGCATGACTGGGAGGCTGGCTACTTT-3'
UM-19	5'-GCCTCTACCAAAAGTAGCCAGTTTGAATGCACGTCGGGAAAC-3'
UM-20	5'-GTGCATTCAAAACCGCTAAAGTTCGTTGTGCGAAGACGACTT-3'
UM-21	5'-GCACAACGAAAGTGAAATGGAGAGGTGAGACTATCACTGCGT-3'
UM-22	5'-AGTCTCACCTCCTCCTGTTGCCTTCACTCCCATGAGTCCTTC-3'
UM-23	5'-GAGGGCAAAGGAAGGACTCATTTATGTCGAGCACGAGTGGAC-3'
UM-24	5'-GCTCGACATAAGCAGATCCACAATCGCGTTCAGTACCAGTTG-3'
UM-25	5'-GGTGTCCCTTCAACTGGTACTCGTAGCTTTACCGATCTAGGC-3'

UM-26	5'-GTAAAGCTACGCTGCCTCGTAACCGCAATCAATTCCTACGTT-3'
UM-27	5'-CCGTATTCCGAACGTAGGAATGAGCTATCCAGATGCGGAGAT-3'
UM-28	5'-CTGGATAGCTCGGCTCCTACCCTACTGCCCAAATCGGACCTA-3'
UM-29	5'-CCTCCCAGTCTAGGTCCGATTCATAGTTCCGTCTTTAGCGGT-3'
UM-30	5'-ACGGAACTATGCTGGGTCACTCGGTCGAAAATCCATTTCACT-3'
UM-31	5'-TTTTCGACCGTTTGAGTGTGTAAGTTTACGGTGCAACAGGAG-3'
UM-32	5'-ACCGTAAACTTCAAGGATGCCGTGGAAGCACTTGTACCTCAG-3'
UM-33	5'-GGGAGTGAAGCTGAGGTACAATCTATCTGCCAGTGGATCTGC-3'
UM-34	5'-TGGCAGATAGAAAGCGTTCTGGACGGTATGGGACTTTGGAGT-3'
UM-35	5'-GAACGCGATTACTCCAAAGTCGAGGACAGAAGTACGAGGCAG-3'
UM-36	5'-CTTCTGTCCTCCGGTGATGAAACATGGCGAAACACTTATGCT-3'
UM-37	5'-TGATTGCGGTAGCATAAGTGTGCAGGTTATCCGGTAGGAGCC-3'
UM-38	5'-GGATAACCTGCGTCTTGCGAAACGATGACCAACCACGAGATA-3'
UM-39	5'-TGGGCAGTAGTATCTCGTGGTACGGGTAATTCAGTGACCCAG-3'
UM-40	5'-GAATTACCCGTGGGGACCATTCTGTACTGCGACACACTCAAA-3
UM42-31	5'-TTTTCGACCGTTTGAGTGTGTAAGTTTACGGTGCAACAGGAG-3'
UM42-32	5'-ACCGTAAACTTGTGCCGTACTCGTGTCAGTGCTGGCAAGAAA-3'
UM42-33	5'-GGGAGTGAAGTTTCTTGCCAGTTAAGAACCCGGTGGATCTGC-3'
UM42-34	5'-CGGGTTCTTAATGCGACGTATCTTCCTGCGATGCTTGAATCT-3'
UM42-35	5'-GAACGCGATTAGATTCAAGCACGAGGGTTAAGTACGAGGCAG-3'
UM42-36	5'-CTTAACCCTCGGAGTGCGGATGCAACGCCTACCTCAATGAAG-3'
UM42-37	5'-TGATTGCGGTCTTCATTGAGGCTCGATAACCTGGTAGGAGCC-3'
UM42-38	5'-AGGTTATCGAGGAGTAAGCGGAGTCAGACGTTAGACGTCCTA-3'
UM42-39	5'-TGGGCAGTAGTAGGACGTCTAGTAACACCAGAAGTGACCCAG-3'
UM42-40	5'-TCTGGTGTTACCGGACATGACGTTTGCGTCAACACACTCAAA-3'
UM42-41	5'-TGACGCAAACCGCATGGTATTCACCAAACATTAGTACGGCAC-3
UM42-42	5'-AATGTTTGGTGCGATACAGCGCCTTGGTTCCATTCTGCCTTA-3'
UM42-43	5'-CACTGACACGTAAGGCAGAATTAGGTAGCACAATACGTCGCA-3'
UM42-44	5'-TGTGCTACCTATGGGTACGAAAGCCGTGTAGTAATCGACTCG-3'

UM42-45	5'-TCGCAGGAAGCGAGTCGATTAGCTATGGTGTGATCCGCACTC-3'
UM42-46	5'-CACACCATAGCCTGCTGTCCACAAAGAGGCGAGTTACAGTGG-3'
UM42-47	5'-TAGGCGTTGCCCACTGTAACTTCCATCTACAGCCGCTTACTC-3'
UM42-48	5'-CTGTAGATGGAGAAGCGGTACCCTGTCTGCAAGGTAACGACC-3'
UM42-49	5'-ACGTCTGACTGGTCGTTACCTACTCAGGTCTTGTCATGTCCG-3'
UM42-50	5'-AAGACCTGAGTTTTGTCGGTCCACGAGACCAAATACCATGCG-3'
UM42-51	5'-TGGTCTCGTGACACTTGTAGCGATGAGAGCCACGCTGTATCG-3'
UM42-52	5'-TGGCTCTCATCCAAGGATGCCGTGGAAGCACGGAGACTTTGA-3'
UM42-53	5'-GGAACCAAGGTCAAAGTCTCCAACAATGGCTGTTCGTACCCA-3'
UM42-54	5'-CAGCCATTGTTAAGCGTTCTGGACGGTATGGGTCGGATATCT-3'
UM42-55	5'-CTACACGGCTAGATATCCGACGAGCGTGATATTGGACAGCAG-3'
UM42-56	5'-ATATCACGCTCCGGTGATGAAACATGGCGAAAGTATAGCAGC-3'
UM42-57	5'-CGCCTCTTTGGCTGCTATACTCACAGAGCTTAGTACCGCTTC-3'
UM42-58	5'-TAAGCTCTGTGGTCTTGCGAAACGATGACCAACACTACCTGA-3'
UM42-59	5'-TGCAGACAGGTCAGGTAGTGTCTTTCAGCTCAGACCGACAAA-3'
UM42-60	5'-TGAGCTGAAAGGGGGACCATTCTGTACTGCGGCTACAAGTGT-3

Strands for Oligo-Myosin	
BG-Cy3-*a'*	5'-/5AmMC6/TTTTTTCGTTATTACTTAGCATTTGG/3Cy3-3'
BG-Cy3-*b'*	5'-/5AmMC6/TTTTTTGATACGCGCCAATCTCTATA/3Cy3-3'
BG-Flex oligo-*a*	5'-AmMC6/TTTTTTTTTTTTTTTTTTTTTCGTTATTACTTAGCATTTGGTT/3Cy3-3'

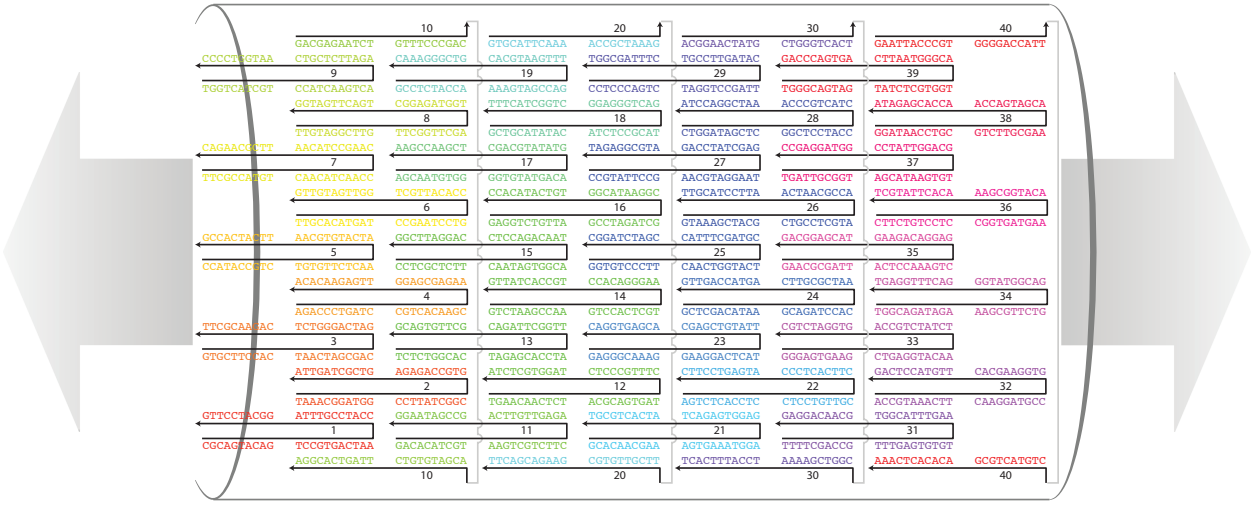
Modified SST for Nanotube Labelling and Myosin Attachment	
UM-11-oligo-*a*	5'–GACACATCGTAAGTCGTCTTCAGAGTTGTTCAGCCGATAAGGTTCCAAA–TGCTAAGTAATAACG–3'
UM-31-oligo-*a*	5'–TTTTCGACCGTTTGAGTGTGTAAGTTTACGGTGCAACAGGAGTTCCAAA–TGCTAAGTAATAACG–3'
UM-31- Flex-oligo-*a*	5'–TTTTCGACCGTTTGAGTGTGTAAGTTTACGGTGCAACAGGAGTTTTTT TTTTTTTTTTTTTTCCAAATGCTAAGTAATAACG–3'
UM-36-biotin	5'–biotin–TTTTCTTCTGTCCTCCGGTGATGAAACATGGCGAAACACTTATGCT–3'
UM42-36-biotin	5'–biotin–TTTTCTTAACCCTCGGAGTGCGGATGCAACGCCTACCTCAATGAAG–3'
UM-18-Cy5	5'–Cy5–TTTTGCTGCATATACATCTCCGCATGACTGGGAGGCTGGCTACTTT–3'

Random DNA mix (42 nt) :

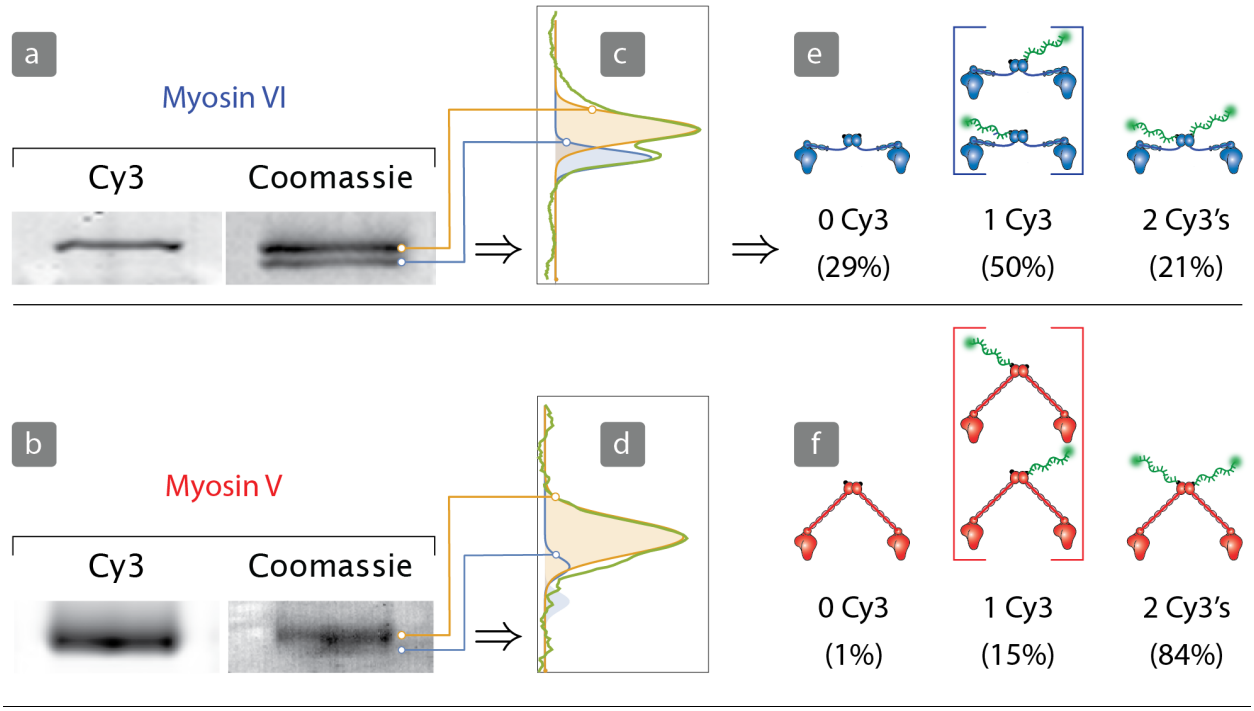
5'–NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN–3'

Supplementary Table 2. Annealing protocol for DNA nanotubes.

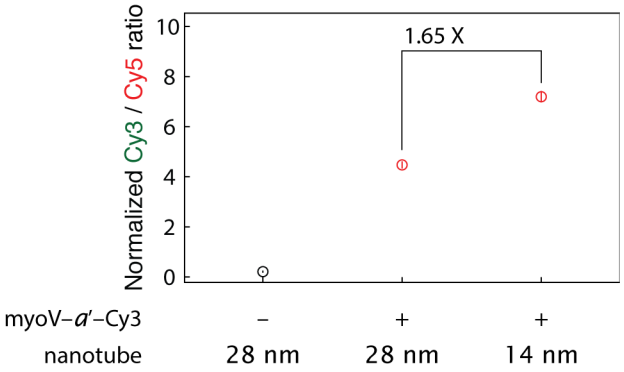
Temperature range	Temperature change	Time interval per temperature change
90-80 °C	0.1 °C	6 sec to 1 min
80-70 °C	0.1 °C	6 sec to 1 min
70-60 °C	0.1 °C	3 min
60-50 °C	0.1 °C	3 min
50-40 °C	0.1 °C	3 min
40-30 °C	0.1 °C	1 min
30-20 °C	0.1 °C	1 min
4 °C	Hold	Hold



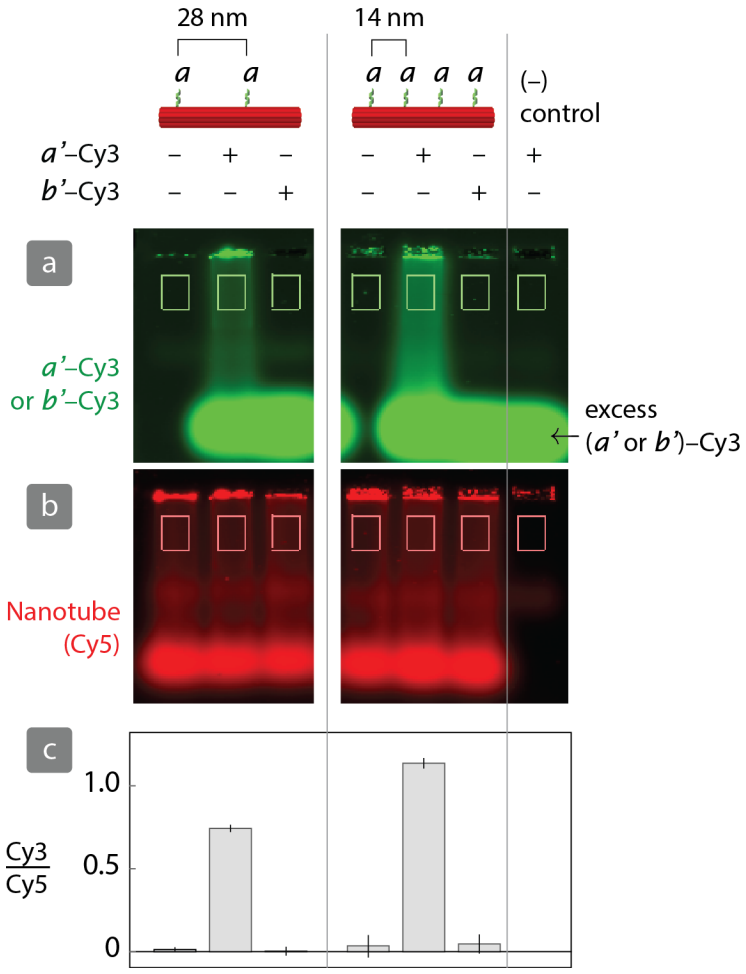
Supplementary Fig. 1 | Design of 10-helix DNA nanotubes with 14 or 28 nm spacing. For the 14 and 28 nm spacing nanotubes, each subunit of the 10-helix DNA nanotube is composed of 40 single-stranded DNA tiles (SST's) (UM01–UM40; Supplementary Table S1). Each SST has four binding domains which uniquely complement with adjacent tiles, and the interaction of which guides nanotube formation.



Supplementary Fig. 2 | Myosin-Cy3 labeling efficiency. (**a**,**b**) Conjugation of Cy3-*a'* to SNAP-tagged myosin VI and V was determined by Coomassie-stained SDS-PAGE gel shift. (**c**,**d**) Myosin-labelling was estimated by fitting the intensity profile of each band along the electrophoresis direction (green). The intensity profile for each condition was derived from the summation of unlabelled (orange) and labelled (blue) myosin monomer, both of which were assumed to be Gaussian distributed. The fraction of population of myosin monomers with 0 and 1 DNA were calculated from the area under the Gaussians. (**e**,**f**) Finally, the fraction of myosin dimers with 0, 1, and 2 DNA strands were estimated from the fraction of myosin monomers with 0 and 1 DNA. In our analysis, we assume that the probability of a labelled myosin monomer being incorporated into a myosin dimer is defined by its binomial distribution. Myosin VI dimer was estimated to be 21% doubly labelled and 50% singly labelled (**e**), whereas myosin V dimer was estimated to be 84% doubly labelled and 15% singly labelled (**f**).



Supplementary Fig. 3 | Summary of normalised myosin V labelling ratios for 14 and 28 nm nanotubes. Ratios were normalised for the myosin Cy3-conjugation efficiency (Supplementary Fig. 2; *N*>140). Except the negative control experiment (left), nanotubes were incubated with myosin V-Cy3- *a'* at room temperature.



Supplementary Fig. 4 | Labelling of DNA nanotubes by agarose gel. (**a**,**b**) 14 and 28 nm nanotubes were labelled with either Cy3 oligo-*a'* or –*b'* and run on a 0.8% agarose gel. Due to the population heterogeneity of nanotube lengths, nanotubes ran as a smear. Intensities of Cy3 (**a**) and Cy5 (**b**) intensities were measured and the Cy3 to Cy5 ratio determined (**c**). 14 and 28 nm nanotubes were specifically labelled with oligo-*a'*, and 14 nm nanotubes had higher labelling that 28 nm nanotubes.

Supplementary Movies

Supplementary Movie 1.
Movie of Alexa-448 phalloidin-labelled actin filaments (green) gliding over myosin VI-labelled 14 nm nanotubes (red). Actin filaments glide specifically along myosin-labelled nanotubes with undetectable non-specific gliding outside the nanotubes.

Supplementary Movie 2.
Simulation of actin movement over a myosin nanotube (N=5 myosin motors). The zero strain position for each myosin is depicted as a grey vertical line. The local tension for each myosin was represented by extension or compression of a spring. In our model, the load for each myosin is always less than stall force (F_{stall}; red horizontal line).

Supplementary Movie 3.
Gliding movement of Alexa-488 phalloidin-labelled actin filaments (green) on human β-cardiac myosin-labelled 14 nm nanotubes (red).