Supporting Information

Lorenz et al. 10.1073/pnas.1003604107

SI Text

Overlay of Myosin II (Postrigor, 2MYS) on Myosin V (Rigor, Chain A of 1W8J). Overlays were calculated from C_α atoms in the residue ranges given below using the program u3best (1).

1. Overlay of lower 50-K domains of 2MYS on 1W8J. Ranges for overlay:

2MYS	Myosin V
525–566	498–539
652–690	639–677
474–485	448–459
579–599	548–568

rmsd on 114 C_α atoms: 0.707 Å. The lower 50-K domains from myosin V and 2MYS are very similar.

Definition of lower 50-K domain for 2MYS:

465–487
517–567
577–600
647–667

2. Overlay of upper 50-K domains of 2MYS and 1W8J. 2MYS was rotated so as to bring the lower 50-K domains into coincidence. Then the overlay necessary to bring the upper 50-K domains into coincidence was calculated.

Ranges for overlay:

2MYS	Myosin 5
420–449	394–423
234–245	208–219
328–363	302–337
382–401	356–375
605–613	574–582
618–622	588–592

rmsd on 112 C_α atoms: 1.012 Å.

The overlay is achieved by rotating the upper 50-K domain of 2MYS through an angle of 22° about the axis depicted in Fig. S2. This axis lies along the β-sheet roughly at right angles to the actin helix axis. Note that the end strands (6th and 7th) of the β-sheet are twisted too much by this procedure.

Definition of upper 50-K domain for 2MYS:

218–465
601–625

3. Overlay of N-C-terminal domains for 1W8J and 2MYS. As above, 2MYS was rotated so as to bring the lower 50-K domains into coincidence. Then the overlay necessary to bring the N-C-terminal domains into coincidence was calculated.

Ranges for overlay:

2MYS	Myosin V
698–708	685–695
132–172	116–156
186–197	170–181

rmsd on 64 C_α atoms: 1.164 Å.

The overlay is achieved by rotating the upper 50-K domain through an angle of 8.1° about the axis depicted in Fig. S3. This axis lies along the β-sheet roughly at right angles to the actin helix axis.

Definition of N-C-terminal domain for 2MYS:

4–199	
488–516	
668–843	
859–1,008	(Regulatory light chain)
1,016–1,160	(Essential light chain)

The converter domain carries the long lever arm of myosin bearing the light chains. The orientation of the converter domain achieved by the process outlined above leads to a positioning of the lever arm that is in excellent agreement with the EM density map.

The structure data generated by imposing these two rotations on the myosin II coordinates (referred to as myo2-5) were used as the starting coordinates for the MD run.

1. Kabsch W (1976) A solution for the best rotation to relate two sets of vectors. *Acta Crystallogr* A32:922–923.

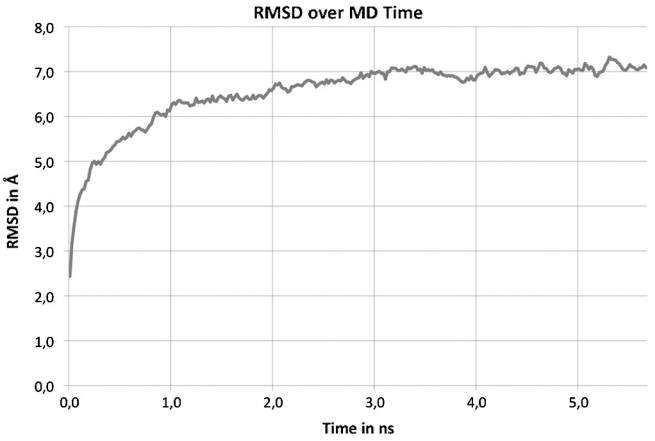

Fig. S1. Backbone rmsd of the final structure compared with the start structure of the actin-S1 complex. Convergence is already achieved after about 2 ns where the actin binding cleft in S1 has closed.

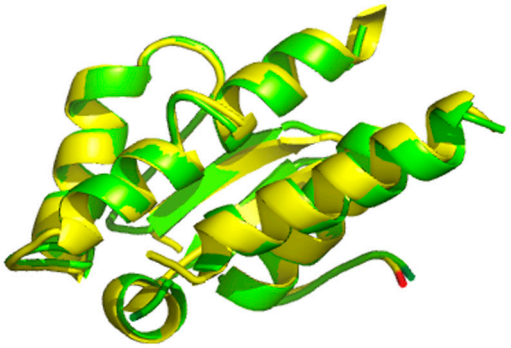

Fig. S2. Secondary structure cartoons showing the precise overlay that can be achieved between the lower 50-K domains of myosin II (2MYS, magenta) and myosin V (light green—chain A of 1W8J). Orientation as in Fig. 3. Generated with PyMol (1).

1. DeLano WL, Lam JW (2005) PyMOL: A communications tool for computational models. *Abstr Pap Am Chem S.* 230:254–COMP.

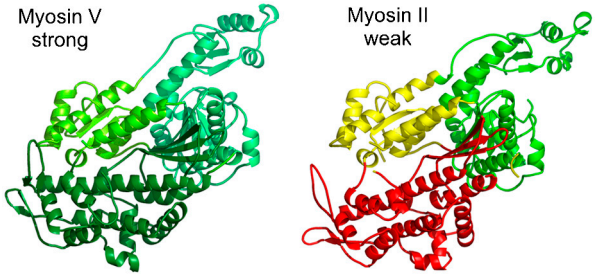

Fig. S3. The domain structure of the myosin head shown as secondary structure cartoons: Myosin 5 (strong binding form) lower 50-K domain—light green, upper 50-K domain—dark green, N-C-terminal domain—apple green (for clarity the N-terminal 60 residues are not shown). Myosin 2 (weak binding form) lower 50-K domain—yellow, upper 50-K domain—red, N-C-terminal domain—green (the N-terminal 70 residues are not shown). Orientation as in Fig. 2. Generated with PyMol (1).

1. DeLano WL, Lam JW (2005) PyMOL: A communications tool for computational models. *Abstr Pap Am Chem S.* 230:254–COMP.

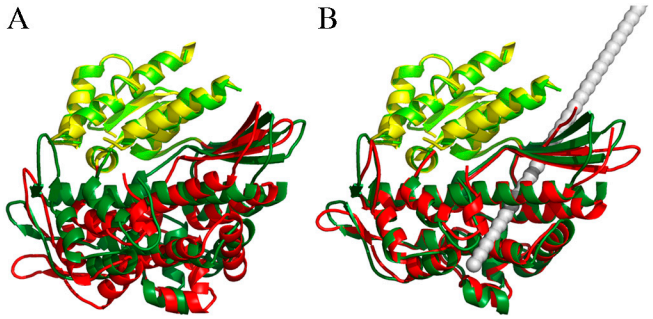

Fig. S4. (*A*) Overlay of the lower 50-K domains (myosin II in magenta, myosin V in light green), upper 50-K domains in red for myosin II, and dark green for myosin V. (*B*) A 22° rotation of the myosin II upper 50-K domain brings it into coincidence with the myosin V upper 50-K domain. The rotation axis is shown (gray). This is approximately at right angles to the actin helix axis and lies roughly in the plane of the β-sheet. (orientation as in Fig S1). Generated with PyMol (1).

1. DeLano WL, Lam JW (2005) PyMOL: A communications tool for computational models. *Abstr Pap Am Chem S.* 230:254–COMP.

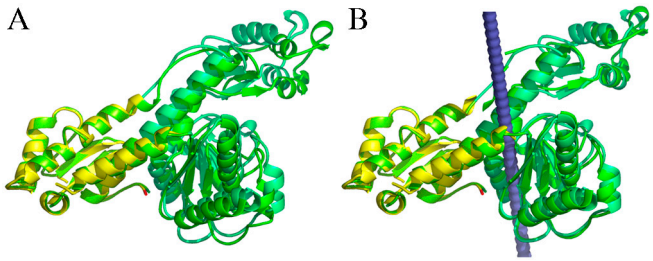

Fig. S5. (*A*) Overlay of the N-C-terminal domains of myosin II (yellow) and myosin V (apple green). Lower 50-K domains colored as in Fig. 4. (*B*) Applying an 8.1° rotation around the shown axis results in an almost perfect overlay of the N-C-terminal domains. The rotation axis—shown in blue—runs roughly along strand 3 of the β-sheet. Generated with PyMol (1).

1. DeLano WL, Lam JW (2005) PyMOL: A communications tool for computational models. *Abstr Pap Am Chem S.* 230:254–COMP.

Table S1. Harmonic constraints for the three runs in molecular dynamics flexible fitting using myo2-5 as a starting model

	Model 1		Model 2		Model 3	
Segment	Sequence	Harm. const.	Sequence	Harm. const.	Sequence	Harm. const.
Actin	7–18	5.0	7–18	5.0	7–18	5.0
	30–37		30–37		30–37	
	52–90		52–90		52–90	
	101–142		101–142		101–142	
	150–240		150–240		150–240	
	247–267		247–267		247–267	
	274–322		274–322		274–322	
	327–329		327–329		327–329	
	336–346		336–346		336–346	
	352–375		352–375		352–375	
ADP (actin)	All	5.0	All	5.0	All	5.0
S1	37–42	1.0		3.0		3.0
	47–53		10–173			
	59–64		191–199		10–199	
	115–119		268–362		219–362	
	123–127		382–402		382–402	
	244–254		418–447		418–474	
	262–269		507–539		507–539	
	278–284		541–557		541–557	
	382–390		581–624		581–624	
	419–452		637–794		637–794	
	475–505					
	689–698					
	178–188	7.0				
	233–244					
	278–284					
	475–506	5.0		5.0		5.0
	718–769		475–506		475–506	
	795–843		795–843		795–843	
ELC/RLC	All	5.0	All	5.0	All	5.0