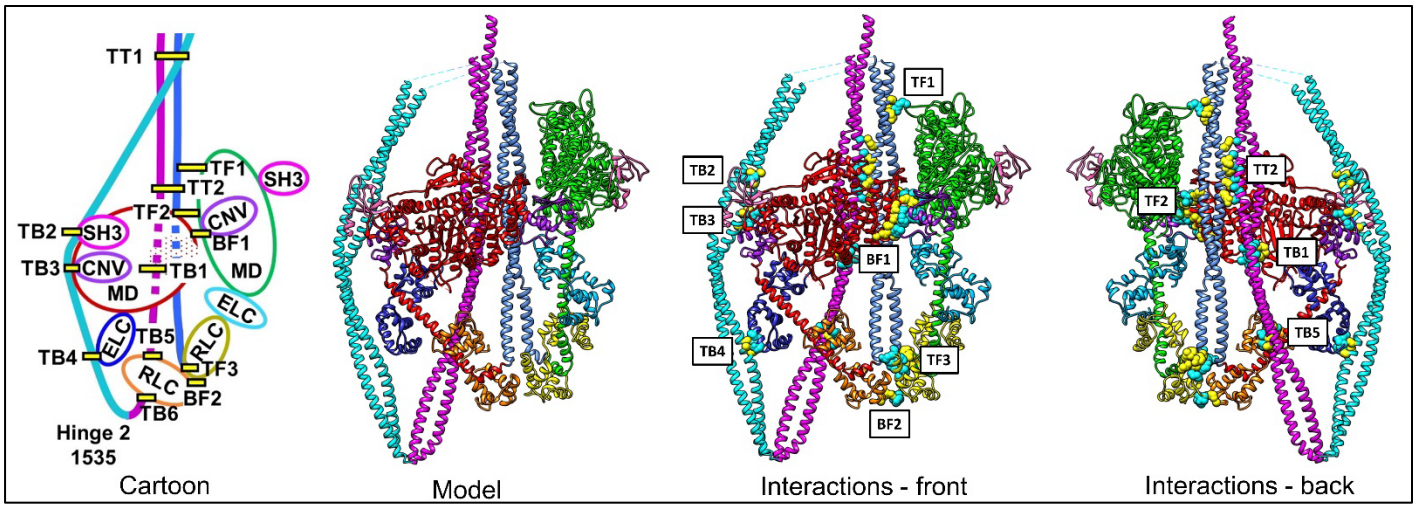
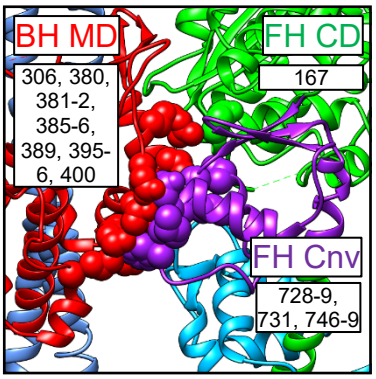
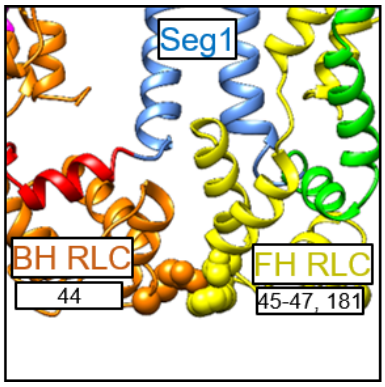
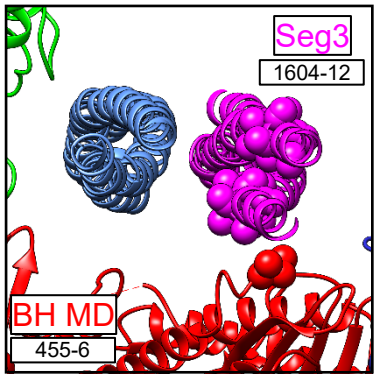
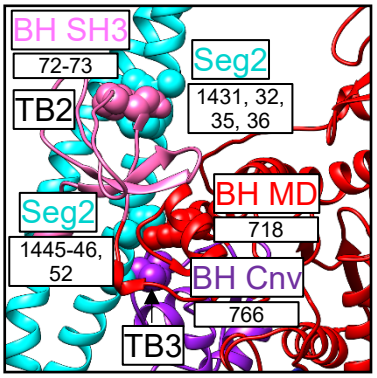
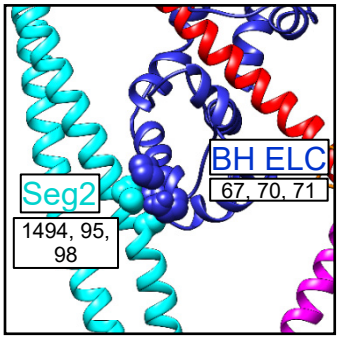
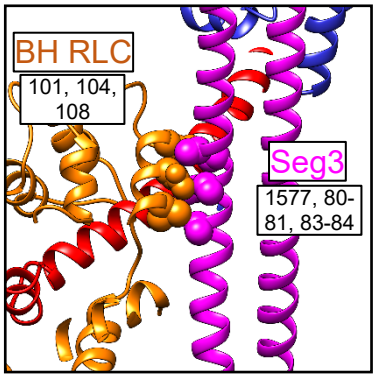
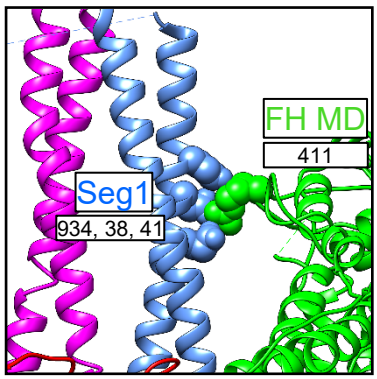
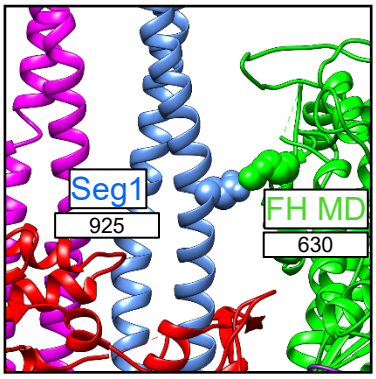
Supplementary Table 1

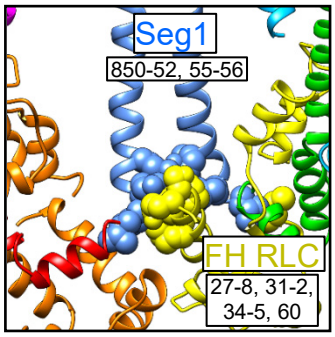
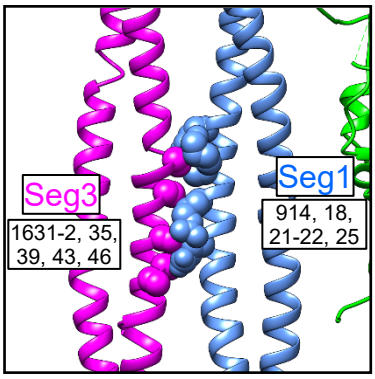
10S myosin intramolecular interactions



Interaction (Ref. 7 nomenclature)	BH Loop/helix (residues)	FH	Segment 1 (S2)	Segment 2	Segment 3	Figure
BF BH/FH **BF1** MD/MD	L306 **C-loop (365-381)** D380 N381 **Helix (382-390)** T382 Q385 K386 H389 **Helix (395-403)** V395	F746 **Helix (727-734)/ Loop (735-748)** D748 D748 G749 R731 **Loop (735-748)** D748 F746 F746 M747 F746 F746 **Helix (729-734)/ Loop (167-170)** Q728				

BF2 RLC/RLC BH RLC/FH RLC PD*	T396 R400 **RLC N-lobe Helix A-helix B linker (41-49)** R44 Q42 R44 E51 D55	Q728 E729 D167 **RLC N-lobe Helix A-helix B linker (41-49)** D45 G46 F47 N81 K8 K8 K12 K12				
TB1 BH MD/segs1,3	**Loop 635-655**† **Loop (450-460)** R455 Q456		**Ring 1**‡ **(~ 912-915?)**		~L1604-E1612	
TB2 BH SH3/seg2	**SH3** K72 D73			Q1432 L1435 D1436 L1431 Q1432 L1435		
TB3 BH Converter/ seg2	**Converter** L766 **Near converter** R718			L1452 Q1445 R1446		
TB4 BH ELC/seg2	**ELC N-lobe D helix (67-77)** E67 L70 P71 E67 E67 L70			L1494 A1495 L1498		

TB5 BH RLC/seg3 **TB6** BH RLC PD/seg3	**RLC C-lobe E helix (99-108)** V101 N104 C108 **RLC PD*** R4 A7 K11 T18 F22				A1577 S1580 Q1581 R1584 E1583 E1572 E1565 L1561 Q1562 E1565 D1566 D1566	
TF1 FH actin-binding loop/segs1,3§		**CM loop (404-420)** R411	**Ring 2**‡ E934‖ E938‖ E941‖			
TF2 FH loop 626-658/segs1,3§		**Loop 2 (626-658)** R630	E925¶			

TF3[#, **] FH RLC/seg1 (there is no interaction with seg3)		**RLC N-lobe A-helix (27-40)** Q27 S28 Q31 E32 K34 E35 M60	 L850 L851 Q852 R855 Q856		No interaction	
TT1 Segs 1, 2, 3 with each other			This region of the 10S molecule is not included in the reconstruction			
TT2 Seg1/seg3 where they cross the BH			E914 R918 A921 K922 E925		D1631 V1632 L1635 L1635 V1639 N1643 N1643 R1646 N1643	

Legend: The table shows potential interactions between different regions of the 10S atomic model, determined using the default parameters of the UCSF Chimera *Find Contacts* tool (proximity of van der Waals surfaces of atoms < 0.4 Å, thus typically center-to-center atomic distances ≤ ~4.2 Å). Because of limited resolution of the reconstruction, the table gives the general region of the contact (loop or helix, in **bold**), with the specific residues detected by Chimera (normal type). These specific contacts should be considered as approximate and provisional. Some loops are missing so there may be more interactions than we have detected. Note that the residue numbers in segments 2 and 3 are not definitive, as they depend on the assumption that hinge 2 occurs at E1535[4], based on analysis of 2D class averages from negative stain data. These hinge locations could be in error by up to 3 amino acids[4]. Tail interactions may be more extensive than we report, as we have not included side-chains on segments 2 and 3—due to their absence from the density map and our uncertainty of the precise longitudinal positioning of the residues in these segments. All interactions are viewed from front except TB1, and TB5. See also Fig. 3 of text. Nomenclature for the different interactions is that defined by Yang et al., 2019[7] (see top panel). The interactions are labeled in the cartoon and shown in the images, with spheres for the residues involved and their numbers in boxes. In the cartoon, **B** = BH; **F** = FH; **T** = tail.

* PD = phosphorylation domain (RLC N-terminal region residues 1-24). Density for the two PDs was less than for the rest of the RLC and so fitting is less certain. The fit is that for the MD simulation structures shown in Extended Data Fig. 8. The best fit positions suggest additional interactions between the RLCs, involving the FH PD (adding to BF2), and between the BH PD and seg3, creating interaction TB6, which would strengthen TB5.

† This loop is omitted from the atomic model as there was not sufficient density to model it accurately. Nevertheless, density in the region of this loop is seen extending from both the BH and FH to seg1, suggesting possible interaction.

‡ Rings 1, 2 and 3 are patches of negative charge in subfragment 2[19].

§ In Yang et al., segs 1 and 3 were not resolved. Our model here shows that only seg1 is involved in interactions TF1 and TF2.

‖ These residues are in the α-helix that is part of the FH HC (chain M in the atomic model)

¶This residue is in the α-helix that is part of the BH HC (chain A in the atomic model)

These potential interactions with the FH RLC occur at the very start of seg1, where the resolution is limiting. They are therefore less certain. The table therefore shows the overall groups of residues involved, without making a specific assignment for each individual interaction.

** In addition to potential interactions with the FH RLC, seg1 also comes close to the BH and FH hooks. As with the interactions with the RLC, the hook interactions are less certain.