S. pombe kinesin-5 switches direction using a steric blocking mechanism

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Supplemental Data

Supplemental Figure S1. Resolution of the cryo-EM reconstruction. Fourier Shell Correlation (FSC) curve computed between two half reconstructions. The FSC 0.5 criterion indicates 9.3 Å resolution (dotted line).

Supplemental Figure S2. Cut7 directional reversal depends on physical crowding. MT gliding driven by Klp2 surfaces, Cut7FL surfaces and Klp2+Cut7 surfaces was compared at different protein concentrations/surface densities and in different buffers. (*A*) 0.35 μM Klp2 surfaces drive MTs to glide with their plus ends leading, indicating that Klp2 motors step towards MT minus ends, as expected, in both KPEM100 and KPEM100 +150 mM NaCl gliding buffers. (*B*) 0.5 μM CutFL surfaces also drive MTs to glide with their plus ends leading under both these conditions. (*C*) A 1.1 μM Cut7FL surface generates plus directed steps in KPEM100. Raising the salt concentration by adding 150 mM NaCl reverts this surface to minus end directed stepping. (*D)* A mixed 0.5 μM Cut7FL and 0.35 μM Klp2 surface steps towards MT minus ends in both KPEM100 + 150 mM NaCl and KPEM + 250 mM NaCl. (*E*) In stark contrast, a mix of 1.1 μM Cut7 and 0.35 μM Klp2 reverses directionality, in both KPEM100 +150 mM NaCl and KPEM + 250 mM NaCl. The data show that a mixed surface of Klp2 and Cut7 converts from net minus end directed stepping to net plus end directed stepping only under crowded conditions. With insufficient crowding, a mixed Klp2 plus Cut7 surface steps towards MT minus ends. This control experiment confirms that only when Klp2 is added to an already-high density Cut7 surface does it cause directional switching.

Supplemental Figure S3. Stepping direction of mixed Cut7FL – dynein MTBD surfaces depends on ionic strength. (*A*) A mixed surface of Cut7FL and dynein MTBD steps towards MT plus ends in KPEM 100. This same surface reverses direction in (*B*) KPEM + 100 mM NaCl and (*C*) KPEM + 200 mM NaCl. Flushing with KPEM 100 (*D*) reverts the surface to plus end directed stepping.

Supplemental Movies

Movie S1. Dissolution of a Cut7-assembled MT bundle by ATP-driven sliding of the component MTs on a Cut7 surface. The bundle was formed in solution by mixing Cut7FL motor with polarity marked MTs and then captured on to a Cut7FL-coated coverslip. The Cut7FL surface then drives the bundled MTs to slide apart. The previously-bundled MTs all slide in the same direction across the coverslip surface, indicating that the MTs were aligned in parallel in the two halves of the bundle. Frame interval 5 s.

Movie S2. Cut7FL-driven sliding of Cut7FL-bundled MTs. Two polarity-marked cargo microtubules (magenta) sliding over two polarity-marked template microtubules (green) that are bound to a neutravidin-coated coverslip via biotin tubulin sparsely incorporated into the green MTs. The cargo MTs slide with their plus ends trailing. Scale bar 5 μm; frame interval 20 s.

Movie S3. Cut7FL-driven sliding of Cut7FL-bundled MTs. A polarity-marked cargo microtubule (magenta) slides on a polarity marked template microtubule (green) bound to a neutravidin-coated coverslip via biotin tubulin sparsely incorporated into the green MTs. The MT slides with its plus end leading. Scale bar 5 μm; frame interval 20 s.

Movie S4. Cut7FL-driven sliding of Cut7FL-bundled MTs. A polarity-marked cargo microtubule (magenta) slides on a polarity marked template microtubule (green) bound to a neutravidin-coated coverslip via biotin tubulin sparsely incorporated into the green MTs. The cargo MT alternates switches its sliding direction repeatedly. Scale bar 5 μm; frame interval 20 s.

Movie S5. Sliding of polarity-marked MTs on a relatively sparse surface of Cut7FL. Conditions: KPEM100 + 100 mM NaCl gliding buffer, 1 mM ATP. MTs slide with their plus ends leading, indicating minus end directed motor stepping. Frame interval 5 s.

Movie S6. Sliding of MTs on a very sparse surface of Cut7FL. Conditions: KPEM100 + 100 mM NaCl gliding buffer, 1 mM ATP. Overlay emphasises repeated pivoting of the sliding MTs at specific points on the surface. The pivoting behaviour suggests that single surface-attached Cut7FL molecules are stepping rapidly and processively towards minus ends. Time code = mins and secs.

Movie S7. Sliding of MTs on a high-density surface of Cut7FL at low ionic strength. Conditions: KPEM100 + 100 mM NaCl gliding buffer, 1 mM ATP. MTs move smoothly and slowly with their plus ends (marked) trailing, indicating plus end directed motor stepping. Frame interval is 5 s.

Movie S8. Cut7FL directionality can be switched by varying buffer conditions. Representative data from a serial wash-through experiment on a single flow cell, corresponding to Fig. 2*C*. A flow cell in

which MTs were gliding on a 1.1 µM Cut7FL surface in KPEM100 gliding buffer, 1 mM ATP (*A*) was serially flushed with (*B*) KPEM100 + 100 mM NaCl gliding buffer, 1 mM ATP (*C*) KPEM100 + 200 mM NaCl gliding buffer, 1 mM ATP (D) KPEM30 gliding buffer, 1 mM ATP.

Movie S9. Sliding of MTs on a surface of Cut7|67-432 monomers. The assay was performed in KPEM30 gliding buffer, 0.1 mM MgATP. At higher ATP concentrations and/or higher salt concentrations, MTs attached only very rarely to the surface. MTs slide exclusively with their plus ends trailing, indicating plus end directed motor activity. Frame interval is 5 s.

Movie S10. Sliding of MTs on a surface of Cut7|1-432 monomers. The assay was performed in KPEM100 gliding buffer, 1mM MgATP. MTs slide exclusively with their plus ends trailing, indicating plus end directed motor activity. Frame interval is 30 s.

Movie S11. Cryo-EM reconstruction with docked pseudo-atomic model of MT-bound *S. pombe* Cut7 motor domain in the AMPPNP state. The N-terminus (blue) is shown in shown in several different possible fits (see text).

Movie S12. Dynein MTBD can crowd Cut7 and reverse its directionality. In KPEM 100 gliding buffer, Cut7FL surfaces (*A*) drive MTs to glide with their plus ends (marked) leading, indicating that the motors on the surface are stepping towards MT minus ends. Adding dynein MTBD to this surface under the same buffer conditions causes the MT gliding direction to reverse. Frame interval 60s.

Movie S13. Klp2 can crowd Cut7 and reverse its directionality. In KPEM100 + 250mM NaCl gliding buffer, 1 mM ATP, a surface made using 1.1µM Cut7FL Cut7FL plus 0.35 µM Klp2 slides MTs with their plus ends trailing, indicating net plus end directed motor stepping (see Fig. *5* and Fig. S2). Plus ends of sliding MTs are marked for clarity. Note that one MT (marked in green) is bidirectional, and that a population of very short MTs moves much more rapidly. We speculate that some surface-tethered Cut7FL tetramers reach above the surface-tethered Klp2 to engage these short MTs. Frame interval 30 s.