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29. J. C. Wu, J. W. Kozarich, J. Stubbe, *J. Biol. Chem.* **258**, 4694 (1983).
30. E. Kühn-Hölsken, C. Lenz, B. Sander, R. Lührmann, H. Urlaub, *RNA* **11**, 1915 (2005).
31. E. C. Small, S. R. Leggett, A. A. Winans, J. P. Staley, *Mol. Cell* **23**, 389 (2006).
32. J. R. Williamson, *Nat. Struct. Biol.* **7**, 834 (2000).
33. A. Schultz, S. Nottrott, N. J. Watkins, R. Lührmann, *Mol. Cell. Biol.* **26**, 5146 (2006).
34. PyMOL, http://pymol.sourceforge.net/.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5821/115/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Tables S1 and S2
References

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An ATP Gate Controls Tubulin Binding by the Tethered Head of Kinesin-1

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Kinesin-1 is a two-headed molecular motor that walks along microtubules, with each step gated by adenosine triphosphate (ATP) binding. Existing models for the gating mechanism propose a role for the microtubule lattice. We show that unpolymerized tubulin binds to kinesin-1, causing tubulin-activated release of adenosine diphosphate (ADP). With no added nucleotide, each kinesin-1 dimer binds one tubulin heterodimer. In adenylyl-imidodiphosphate (AMP-PNP), a nonhydrolyzable ATP analog, each kinesin-1 dimer binds two tubulin heterodimers. The data reveal an ATP gate that operates independently of the microtubule lattice, by ATP-dependent release of a steric or allosteric block on the tubulin binding site of the tethered kinesin-ADP head.

Kinesin-1 molecular motors are adenosine triphosphate (ATP)–driven walking machines that move in 8-nm steps toward the plus ends of microtubules, turning over one ATP molecule per step under a range of loads (*1*–*5*). Even at very high backward loads, when the motor can be forced to step processively backward (*6*), stepping remains coupled to ATP binding (*6*, *7*). Between steps, the motor pauses stably in a dwell state. It is clear that ATP binding triggers exit from this dwell state, but the structural mechanism is controversial (*8*).

Two general types of model for this ATP gate have been proposed. In the first, kinesin dimers are proposed to dwell between steps with only one head attached to the microtubule, whereas the other diffuses to some extent on its tether but cannot access its next binding site along the microtubule because the site is too far away. ATP binding to the microtubule-attached head drives a conformational change that shifts the tethered head along the microtubule, biasing and focusing its diffusional search for its next binding site (*9*, *10*). In the second type of model, kinesin is proposed to dwell with both heads attached to the microtubule (*5*), and gating is ascribed to the effects of the resulting intramolecular strain, together with any external strain, on nucleotide exchange (*11*, *12*). These two types of model are not mutually exclusive; the influential Rice *et al*. model (*9*), for example, proposes that the first step in each run of steps uses the first type of gate and that subsequent steps use the second type. Both types of model require the microtubule lattice for their operation, either to set a prohibitive distance between binding sites or to apply strain to the kinesin heads. Here, we report an ATP gate that operates independently of the microtubule lattice.

We have found that kinesin-1 binds to free tubulin heterodimers in solution, causing tubulin-activated release of adenosine diphosphate (ADP). This shows that tubulin activation of the kinesin adenosine triphosphatase (ATPase) is not unique, as had previously been thought, to the depolymerizing kinesins (*13*). The degree of activation of the kinesin-1 ATPase by unpolymerized tubulin varies according to the source of kinesin-1 and tubulin, but clearly activation of the kinesin-1 ATPase does not require the interheterodimer interfaces that arise in the microtubule lattice. For a fungal kinesin-1 and a fungal tubulin, maximal activation by unpolymerized tubulin heterodimers is equivalent to that produced by assembled microtubules (Fig. 1A). For brain tubulin and brain kinesin, tubulin activation of the kinesin ATPase is modest compared with microtubule activation (Fig. 1, B to D).

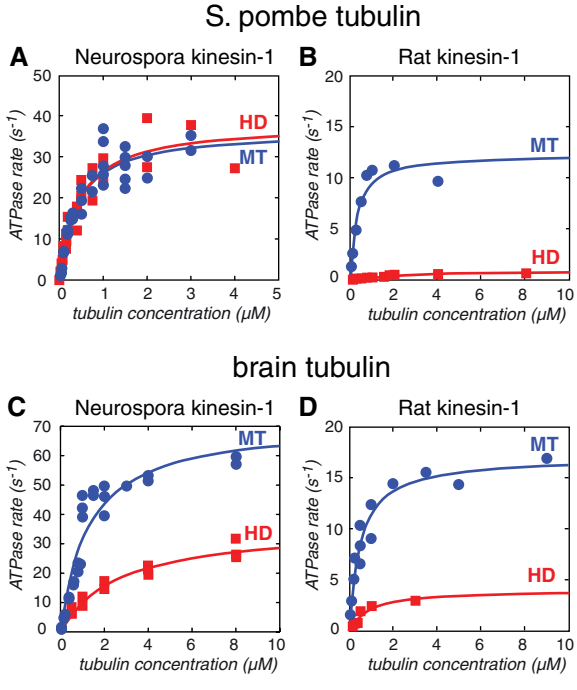

Fig. 1. Activation of kinesin dimers by tubulin and microtubules. The microtubule- or tubulin heterodimer–stimulated steady state ATPase activity of kinesin was measured at 25°C with an enzyme-linked assay in 20 mM Pipes, pH 6.9, 5 mM $MgCl_2$, 1 mM dithiothreitol (*22*). Values for V_{max} (the projected maximum ATPase rate) and K_m (the tubulin concentration giving half-maximal ATPase) were obtained by least-squares fitting to plots of ATPase versus tubulin heterodimer concentration, using Kaleidagraph 3.6.4 (Synergy Software, Reading, PA, USA). MT, microtubule; HD, heterodimer. (**A**) V_{max} 38.1 s^{-1}, K_m 0.44 μM for HD, V_{max} 36.4 s^{-1}, K_m 0.39 μM for MT. (**B**) V_{max} 0.9 s^{-1}, K_m 2.03 μM for HD, V_{max} 12.2 s^{-1}, K_m 0.28 μM for MT. (**C**) V_{max} 35.9 s^{-1}, K_m 1.29 μM for HD, V_{max} 71.1 s^{-1}, K_m 0.62 μM for MT. (**D**) V_{max} 4.0 s^{-1}, K_m 0.86 μM for HD, V_{max} 17.0 s^{-1}, K_m 0.49 μM for MT.

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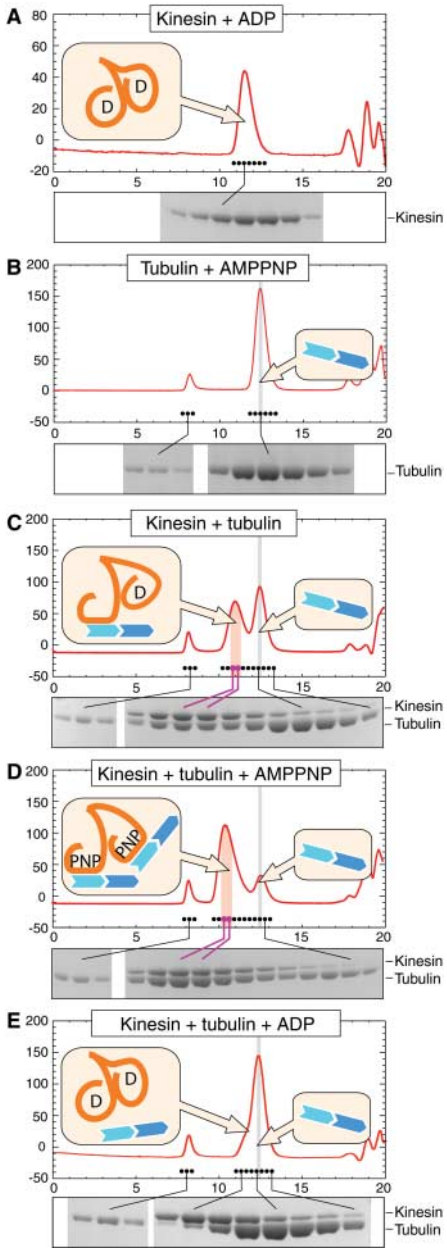

Fig. 2. Superose 12 column chromatography of kinesin-tubulin complexes. (**A**) 6.5 μM rK430 rat kinesin in ADP. (**B**) 13 μM pig-brain tubulin in AMP-PNP. (**C**) 13 μM tubulin + 6.5 μM kinesin; no added nucleotide. (**D**) 13 μM tubulin + 6.5 μM kinesin in 0.2 mM AMP-PNP. (**E**) 13 μM tubulin + 6.5 μM kinesin in 2 mM ADP. *Y*-axis marks are in mAU at 290 nm. *X*-axis marks are at intervals of 1 ml. The included volume of the column was 20.0 ml, and the void volume was 8.1 ml. Samples (240 μl) were run at 0.5 ml min^{-1} in 50 mM Pipes pH 6.9, 2 mM $MgCl_2$, 1 mM EGTA with or without 2 mM ADP or 0.2 mM AMP-PNP. The gray vertical line indicates the tubulin elution position. The elution profiles of tubulin alone and kinesin alone were the same in ADP or AMP-PNP. Binding stoichiometry was measured for the shaded fractions.

Gel-filtration experiments (Fig. 2) revealed that the stoichiometry and affinity for kinesin-1 binding to unpolymerized tubulin depends on the bound nucleotide. With no added nucleotide, kinesin dimers bind tightly to tubulin heterodimers, and the two proteins elute together from a fast protein liquid chromatography gel-filtration column as a complex in which each kinesin dimer binds 1.01 ± 0.06 SD (n = 4) tubulin heterodimers (Fig. 2C). By contrast, in the presence of the nonhydrolyzable ATP analog adenylyl-imidodiphosphate (AMP-PNP), each kinesin dimer binds 1.83 ± 0.08 SD (n = 4) tubulin heterodimers (Fig. 2D). In the presence of ADP, the binding is weakened, although some interaction is still apparent (Fig. 2E). Kinesin binding does not deplete the total amount of tubulin included by the column, even in AMP-PNP, which indicates that kinesin binding does not cause tubulin to aggregate. Furthermore, addition of guanosine monophosphate–carboxypiperazin-4-yl-propyl-1-phosphonic acid (GMP-CPP) or taxol to AMP-PNP–kinesin-tubulin complex did not cause microtubule assembly, as judged by video-enhanced differential interference contrast microscopy, suggesting that the two tubulin heterodimers in the AMP-PNP–kinesin-tubulin complex are held in an arrangement that prohibits their assembly into a microtubule.

Tubulin activation of the kinesin ATPase occurs, as for microtubule activation, by acceleration of the ADP release step of the kinetic cycle (Fig. 3). Using kinesin in which both heads are primed with the fluorescent analog 2′(3′)-O-(N-methylanthraniloyl)-ADP (mantADP), we find that half of the fluorescence signal corresponding to bound mantADP decays on initial mixing with an excess of tubulin heterodimers, whereas the remainder decays only on addition of an AMP-PNP or ATP "chase" (Fig. 3B and 3D). This shows that only one head releases mantADP immediately and that tubulin-activated mantADP release from the second kinesin head requires that AMP-PNP or ATP bind to the first head. This two-step tubulin-activated release of ADP from kinesin is likely to be related to the half-site ADP release for kinesin binding to microtubules first reported by Hackney (*14*, *15*), in which microtubule-activated ADP release occurs from only the microtubule-bound head, whereas ADP release from the tethered head is dependent on the binding of AMP-PNP or ATP to the microtubule-bound head (*16*–*18*). A similar structural mechanism may underlie both behaviors.

How might ATP binding to the microtubule-bound head convert the tethered head from a refractory state in which it traps ADP (*19*) and cannot bind tubulin into a state in which it binds tubulin and releases ADP? Strain-based mechanisms are ruled out, because ATP gating occurs with unpolymerized tubulin. There are then two broad possibilities: a steric mechanism, in which the tubulin binding site in the tethered ADP head is physically masked, or an allosteric mechanism, in which the tubulin binding site on the tethered ADP head undergoes a conformational change that is triggered by ATP binding to the tubulin-attached, nucleotide-free head. To try to distinguish these possibilities, we fitted 3KIN, the only available kinesin dimer crystal structure, into an existing cryogenic electron microscopy (cryo-EM) reconstruction of the complex of kinesin dimers with helical (15 protofilament) microtubules (*20*, *21*), obtained in the absence of added nucleotide. Fitting was only possible by docking each head separately into the cryo-EM density. In the resulting fit, the second kinesin head sits slightly ahead and to the

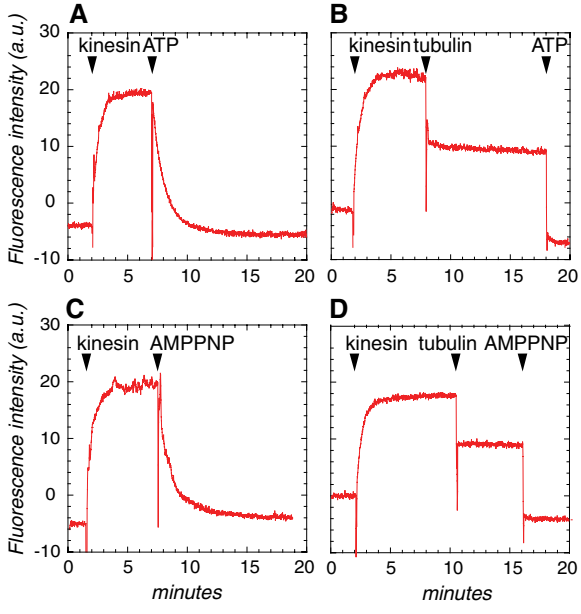

Fig. 3. Two-step tubulin-activated ADP release from kinesin. (**A** and **C**) Fluorescence transients corresponding to slow binding of 1 μM mantATP to 1 μM rat kinesin, followed by slow release of mantADP from both kinesin heads induced by a chase of nonfluorescent 1 mM ATP or 1 mM AMP-PNP. (**B** and **D**) The same experiment, but with 2 μM tubulin heterodimers added before the addition of the chasing nucleotide. Buffer 20 mM Pipes, pH 6.9, 2 mM $MgCl_2$.

left of the attached head (Fig. 4) (*22*), suggesting that the tethered head is positioned so as to mask its microtubule binding site. Further work will be required to test this preliminary interpretation.

Existing data indicates that ATP binding has profound effects on the kinesin head, reconfiguring the active site and shifting the conformation of the microtubule binding loops L8 and L12 and the neck linker. The neck linker is a 13-residue sequence that connects the coiled-coil tail of kinesin-1 to the C terminus of the alpha-6 helix in the head domain. Mutating the neck linker or cross-linking it to the main part of the head inhibits kinesin-driven motility (*23*–*25*). Rice and colleagues (*9*) first proposed that ATP binding to the kinesin head drives an undocked-to-docked transition of its neck linker, and that this event drives stepping of two-headed molecules by shifting the leading head closer to its next binding site along the microtubule. EM evidence from gold-labeled kinesin monomers (*9*), electron paramagnetic resonance studies (*9*, *26*), and a variety of subsequent evidence from fluorescently labeled kinesin dimers (*27*–*29*) is consistent with ATP-dependent neck-linker docking. Our own data show that ATP binding to a tubulin-attached head releases the other head from a sterically or allosterically blocked state. Apparently, ATP binding drives both neck-linker docking and escape of the tethered kinesin head from a blocked state. Further work will be necessary to determine whether these two events are coupled.

In the one-head-blocked kinesin dimer state that we have identified, one head binds tubulin and the other has its tubulin binding site blocked. It is not clear whether this functional asymmetry is due to a preexisting structural asymmetry or whether blocking of one head results from tubulin binding to the other head. In 3KIN, there is already an asymmetry, but modeling indicates that both tubulin binding sites in 3KIN could be occupied without producing a steric clash (*22*). Notwithstanding this issue, and whether the blocking mechanism is steric or allosteric, our data identify an ATP gate that operates independently of the microtubule lattice, by a mechanism that is not based on the strain developed between two attached heads, or on a step-change in the diffusional distance to the next binding site along the microtubule. What role might this gate play in the kinesin walking mechanism?

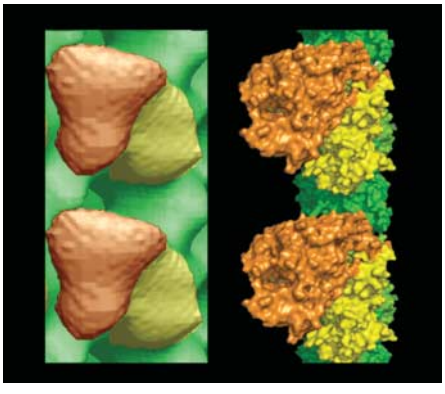

Fig. 4. Fitting of cryo-EM maps of the apo state of kinesin dimers attached to microtubules. The microtubule plus end is toward the top of the page. (**Left**) Cryo-EM map (*20*). (**Right**) Fitted orientation of two heads of rat kinesin. One head (yellow) is attached to the underlying microtubule protofilament, whereas the other head (orange) is parked in a forward-biased position that masks its tubulin binding site.

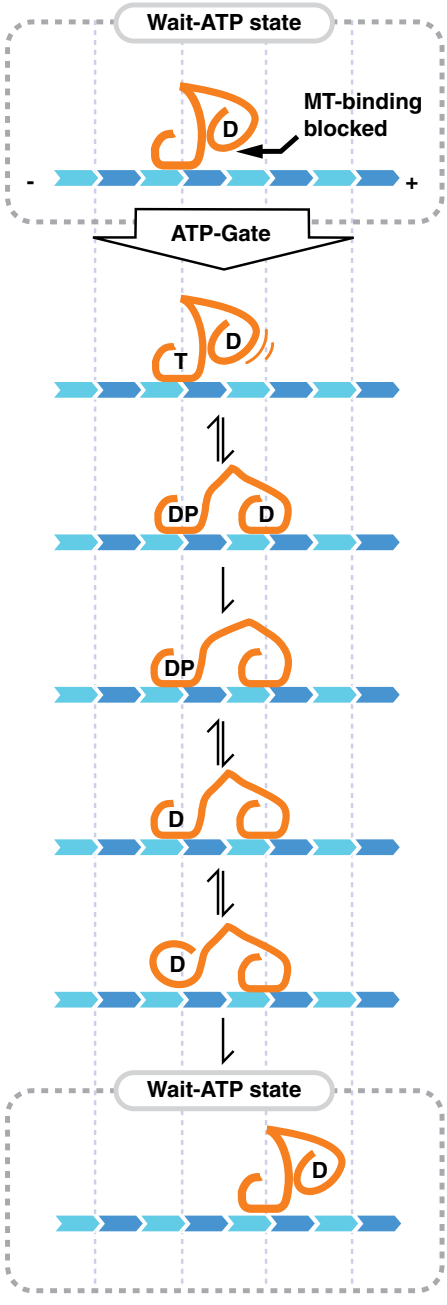

Fig. 5. Gating scheme. The cycle begins with ATP-gated exit of the tethered head from a refractory dwell state (dotted box) in which the tubulin binding site of the tethered head is blocked. After ATP binding to the attached head, the block is released and the tethered head is then free to diffuse to its next site along the microtubule. Hydrolysis and phosphate release on the trailing head return it to a weak-binding K.ADP state, which then detaches, diffuses to a forward-biased position, and reverts to a blocked state.

Our data show that in the absence of ATP, only one head binds tubulin and that ATP binding to this tubulin-attached head is required to unblock the tubulin binding site on the other head. On this basis, we predict the scheme shown in Fig. 5, in which whenever the trailing kinesin head cycles through to the K.ADP intermediate, it detaches and reverts to a state in which its tubulin binding site is blocked. Exit from this blocked state requires ATP binding and is expected to be rate limiting at low ATP concentrations and/or high loads. At high ATP concentrations and low loads, ATP will bind rapidly, and exit from the blocked state will be correspondingly fast. In these circumstances, it is possible that the two-heads-attached configuration will have the longest lifetime in the cycle. Nonetheless, we emphasize that the ATP gate will still operate, requiring that ATP must bind to sanction stepping, and fulfilling its function of checking and adjusting the phasing of the kinetic cycles on the two heads.

At least one current model proposes that the gate controlling kinesin's first step is different from that controlling subsequent steps (*9*). In our model, the same gate controls the first and all subsequent steps. Our model is consistent with recent single-molecule work showing that ATP binding under load is necessary to escape a cycle of repeated futile back-stepping induced by a slowly releasing phosphate analog (*12*) and with earlier work showing that ATP binding is necessary for both foresteps and backsteps (*6*, *7*). Our model is, however, inconsistent with proposals that in the ATP-waiting dwell state, both kinesin heads attach stably to the microtubule (*30*).

A key point of controversy in the kinesin mechanism is the question of which biochemical step or steps generate force. In our model, ATP binding generates force, but indirectly, by sanctioning binding of the ADP-containing lead head to its next site, which in turn triggers microtubule-activated ADP release from the lead head and stabilizes it in a force-holding state (*6*, *8*). The requirement that ATP must bind to allow exit from this state then serves to coordinate lead-head attachment and trail-head detachment and to maintain tight coupling by ensuring a consistent phase lag between the kinetic cycles of the two heads (*16*). An emerging general theme for mechanochemical nucleotidases such as kinesin and myosin is the amplification of local, nucleotide-dependent conformational changes by causing them to gate larger-scale diffusional motions (*8*).

References and Notes

1. J. Howard, A. J. Hudspeth, R. D. Vale, *Nature* **342**, 154 (1989).
2. W. Hua, E. C. Young, M. L. Fleming, J. Gelles, *Nature* **388**, 390 (1997).
3. K. Kaseda, H. Higuchi, K. Hirose, *Nat. Cell Biol.* **5**, 1079 (2003).
4. C. L. Asbury, A. N. Fehr, S. M. Block, *Science* **302**, 2130 (2003).
5. A. Yildiz, M. Tomishige, R. D. Vale, P. R. Selvin, *Science* **303**, 676 (2004).
6. N. J. Carter, R. A. Cross, *Nature* **435**, 308 (2005).
7. M. Nishiyama, H. Higuchi, T. Yanagida, *Nat. Cell Biol.* **4**, 790 (2002).
8. N. J. Carter, R. A. Cross, *Curr. Opin. Cell Biol.* **18**, 61 (2006).
9. S. Rice *et al.*, *Nature* **402**, 778 (1999).
10. W. H. Mather, R. F. Fox, *Biophys. J.* **91**, 2416 (2006).
11. S. S. Rosenfeld, P. M. Fordyce, G. M. Jefferson, P. H. King, S. M. Block, *J. Biol. Chem.* **278**, 18550 (2003).
12. N. R. Guydosh, S. M. Block, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 8054 (2006).
13. C. E. Walczak, *Nat. Cell Biol.* **8**, 903 (2006).
14. D. D. Hackney, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6865 (1994).
15. D. D. Hackney, *Biochemistry* **41**, 4437 (2002).
16. Y. Z. Ma, E. W. Taylor, *J. Biol. Chem.* **272**, 724 (1997).
17. S. P. Gilbert, M. L. Moyer, K. A. Johnson, *Biochemistry* **37**, 792 (1998).
18. I. Crevel, N. Carter, M. Schliwa, R. Cross, *EMBO J.* **18**, 5863 (1999).
19. R. A. Cross, *Trends Biochem. Sci.* **29**, 301 (2004).
20. K. Hirose, J. Lowe, M. Alonso, R. A. Cross, L. A. Amos, *Mol. Biol. Cell* **10**, 2063 (1999).
21. R. A. Cross *et al.*, *Philos. Trans. R. Soc. London B Biol. Sci.* **355**, 459 (2000).
22. Materials and methods are available as supporting material on *Science* Online.
23. M. Tomishige, R. D. Vale, *J. Cell Biol.* **151**, 1081 (2000).
24. R. B. Case, S. Rice, C. L. Hart, B. Ly, R. D. Vale, *Curr. Biol.* **10**, 157 (2000).
25. K. Hahlen *et al.*, *J. Biol. Chem.* **281**, 18868 (2006).
26. S. Rice *et al.*, *Biophys. J.* **84**, 1844 (2003).
27. S. S. Rosenfeld, G. M. Jefferson, P. H. King, *J. Biol. Chem.* **276**, 40167 (2001).
28. K. Sugata, M. Nakamura, S. Ueki, P. G. Fajer, T. Arata, *Biochem. Biophys. Res. Commun.* **314**, 447 (2004).
29. A. B. Asenjo, Y. Weinberg, H. Sosa, *Nat. Struct. Mol. Biol.* **13**, 648 (2006).
30. A. Yildiz, P. R. Selvin, *Trends Cell Biol.* **15**, 112 (2005).

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