# Kinesin Walks Hand-Over-Hand

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## Supplementary Online Material

### Materials and Methods

**Kinesin preparation and labeling**. Truncated "cys-light" human ubiquitous kinesin dimers, containing 560 residues per monomer (340 amino acids (a.a.) per head and 220 a.a. of the coiled-coil), with all solvent-exposed cysteines replaced, were used as a template for mutagenesis (*1*). Homodimers containing a unique reactive cysteine on each head at position E215C or T324C were made using QuikChange mutagenesis. In addition, a heterodimeric kinesin was made containing one head without any solvent-exposed cysteines and the other head containing S43C and T324C (double-cys heterodimer purification protocol will be published elsewhere, Tomishige M., Vale R. D. manuscript in preparation). Kinesin proteins were expressed and purified as described previously (*2*). Eluent from HiTrap-Q column was then dialysed against a buffer (25 mM Pipes [pH 7.0], 100 mM NaCl, 1 mM EGTA, 2 mM $MgCl_2$, 50 µM ATP) for 4 hr at 4ºC. Protein concentration was determined by Bradford assay using BSA as a standard. Dialysed kinesin was reacted with Cy3-maleimide (Amersham Biosciences) at a molar ratio of 0.4 dyes per head for 4 hr at 4ºC for the homodimers and 10 dyes per head for the heterodimer. Unreacted dyes were quenched by adding DTT to a final concentration of 1 mM, and then removed through microtubule affinity purification as described (M. Tomishige, ibid.), except that an ATP concentration of 100 µM was used instead of 5 mM for releasing motors from microtubules. Under these conditions, the vast majority of homodimeric kinesins had only one dye bound. The heterodimers were 1.5-2X brighter than singly labeled homodimers suggesting that two dyes were bound under these labeling conditions.

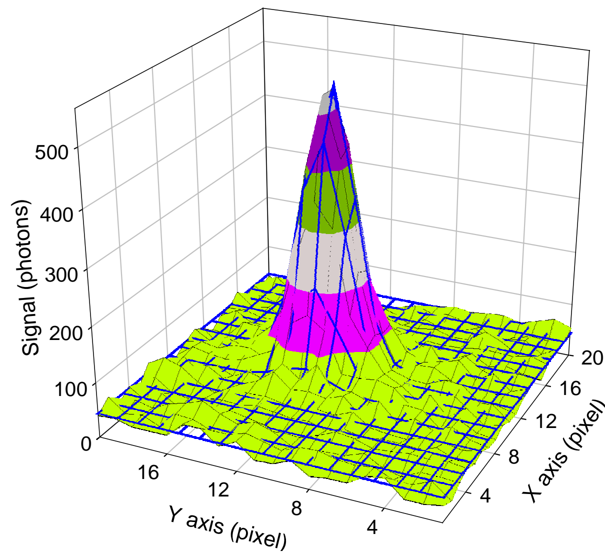
**Axoneme preparation, immobilization, and motility conditions**. Axonemes were prepared from sea urchin as described by Gibbons and (*3*). Axonemes were diluted 4X in BRB12 Buffer (12 mM PIPES pH 6.8, 1 mM EGTA, 2 mM $MgCl_2$) and immobilized onto glass surface by flowing over a glass coverslip in a flow chamber (*4*). The chamber was then inverted and put on ice for 5 min. The sample was then washed with 50 µl BRB12. Kinesin (500 pM) in assay buffer was then perfused into the chamber. {Assay buffer: 2.5 mg/ml casein, 0.4% glucose, ATP regenerase (2 Units/ml creatine kinase, 2 mM creatine phosphate), 15 mM $MgCl_2$, 2 mM DTT, 1.5 µl "gloxy", where gloxy is described in (*4*)}. TIRF microscope is as described previously (*4*). Fluorescence images were collected at 3 mW excitation power at 532 nm, with 0.33 sec per frame. Positional stability measurements of labeled kinesin bound to axonemes in the absence of ATP showed that axonemes and labeled kinesins were immobile, indicating that the axonemes were well stuck to glass surface and kinesins were strongly bound to axonemes. In the absence of ATP, no steps were observed.

**References**

1. S. Rice *et al.*, *Nature* **402**, 778 (1999).
2. M. Tomishige, R. D. Vale, *J. Cell Biol.* **151**, 1081 (2000).
3. I. R. Gibbons, and E. Fronk, *J. Biol. Chem.* **254**,187-196 (1979)
4. A. Yildiz *et al.*, *Science* **300**, 2061 (2003).

**Fig. S1.** (**A**) Point spread function with 0.33 sec integration time of Cy3-labeled kinesin on axoneme (blue trace, Fig 2). A 2-D Gaussian curve (solid blue lines) fits well to the PSF ($r^2$ = 0.984, $\chi^2$=1.25). The number of collected photons is ~6800. The full-width-half-max of the PSF is 288 nm and the signal-to-noise ratio is 25, enabling to the center to be localized to within ± 1.7 nm. (**B**) The intensity at peak pixel vs. time for the single Cy3-kinesin PSF shown in S1A. The Cy3 lasted 20 seconds producing 60 images; single step photobleaching observed.

**A**




**B**

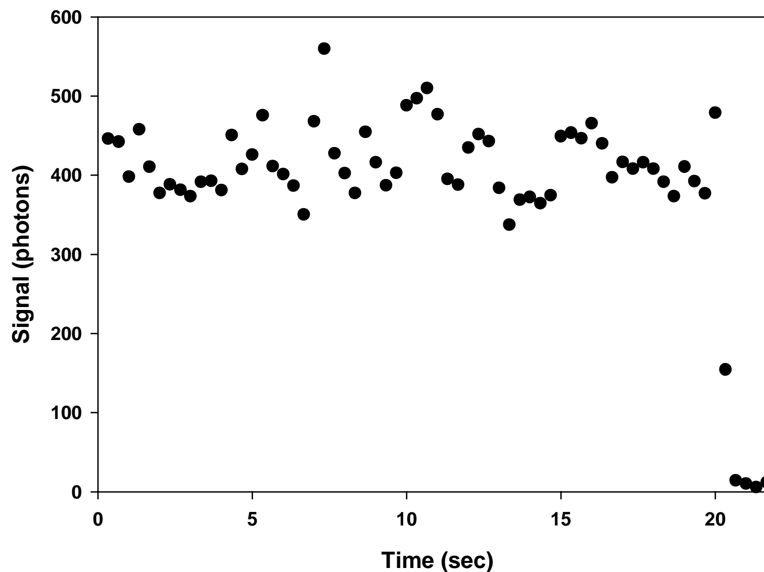


**Fig. S2. Stage stepping data.** The following control experiment was done to show that the rise near t=0 in the dwell time histogram in Fig. 3B is due to a convolution of alternating 0 nm and 17 nm step and not due to an instrument artifact that artificially depressed the values near t=0. Fluorescent beads were immobilized at low density onto a coverslip and the coverslip was moved with a nanometric stage with a rate that was set to 0.59 steps/sec with a step size of 17 nm. (**A**) Trace of position vs. time **(B)** Dwell time histogram showing the expected exponential decay with a maximum near t=0. The best fit was achieved with k=0.65 steps/sec, $r^2$=0.98.

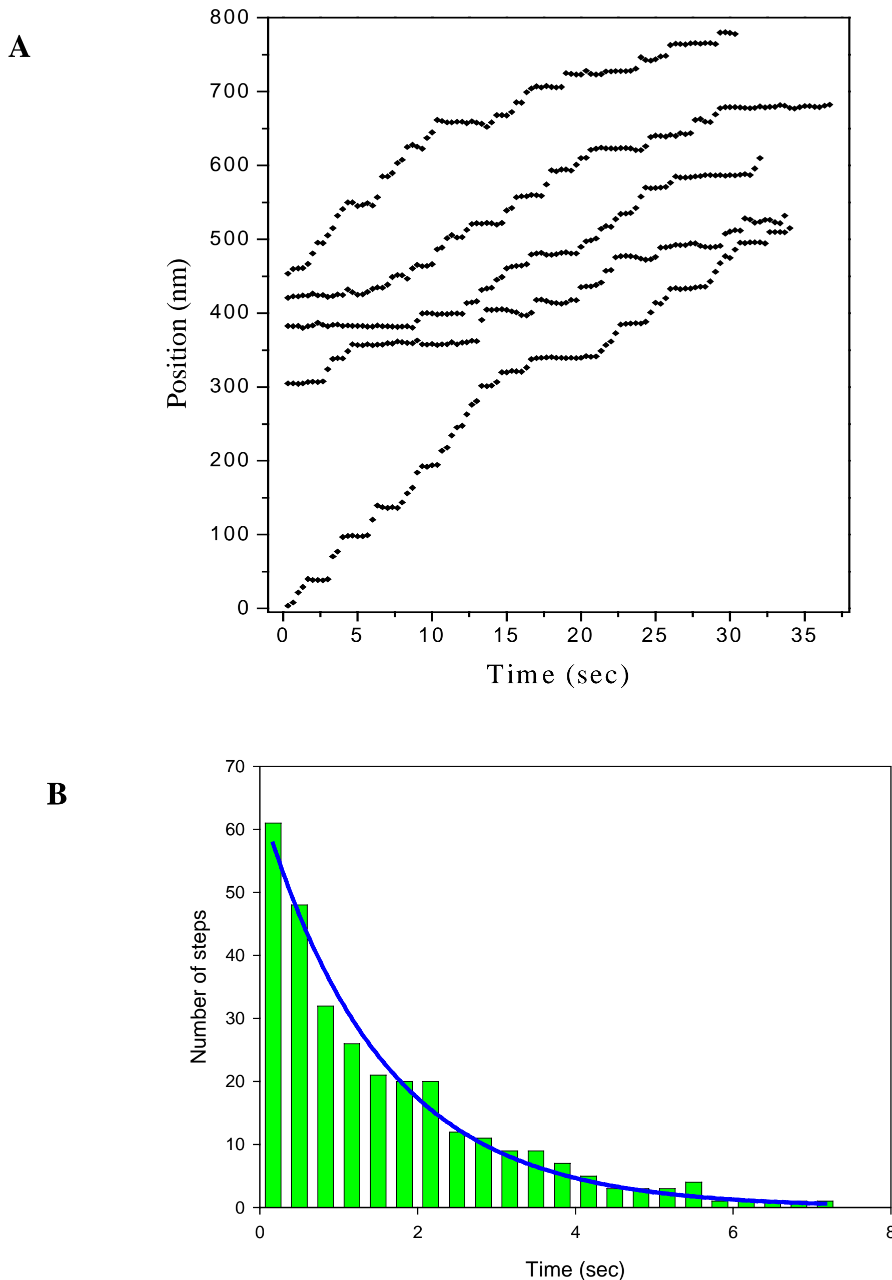
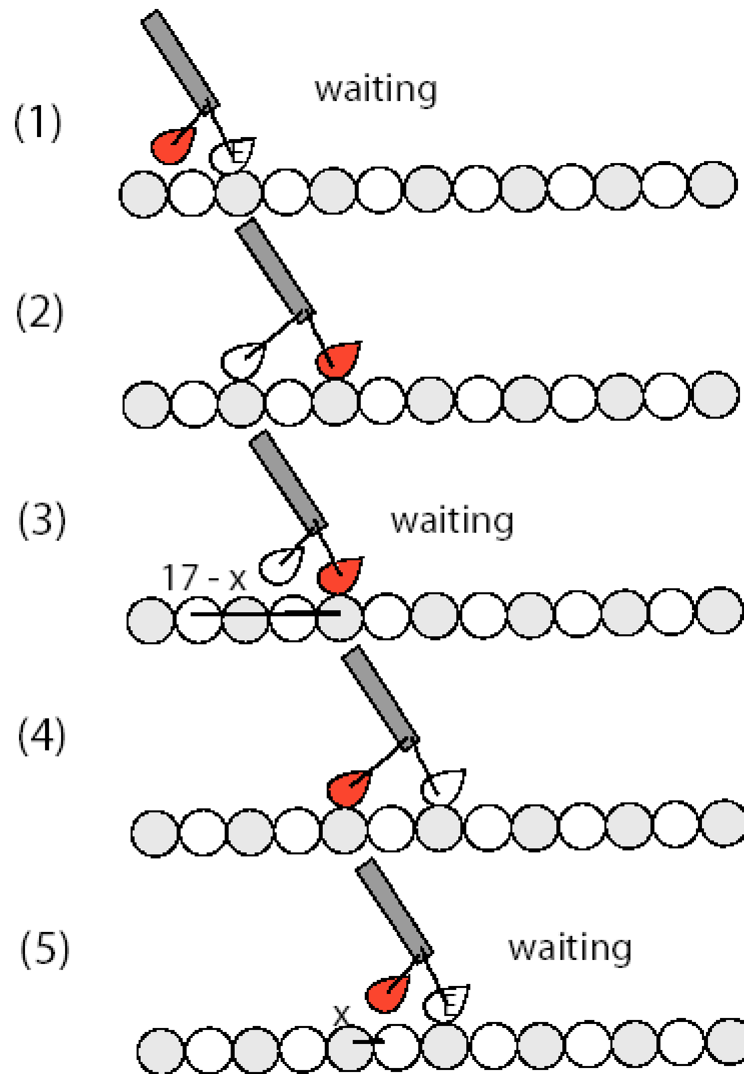


**Fig. S3.** Model for expected step size for a one-headed bound waiting state of kinesin. The step size would alternate between 17- $x$ and $x$, where $x$ is the distance along the direction of motion of the dye from where the dye would be if both heads were bound. Figure courtesy of Yoshi Oono (Univ. of Illinois, Urbana-Champaign).




**Movie M1. A single kinesin molecule moving on an immobilized axoneme.** Movement of a dye on one head of a kinesin dimer along an immobilized axoneme. Cy3 fluorophore was attached to E215C and visualized using total internal reflection fluorescence microscopy. The movie was taken at 340 nM ATP concentration, 0.33 sec integration time per image. The pixel size was 86.6 nm. This spot lived 20 sec producing 60 images (Fig. S1A). The PSF fit well to 2-D Gaussian function revealing the steps of an individual head of kinesin dimer. Tracking of this spot is shown in blue trace in Fig. 2.