

## Supplementary Information for:

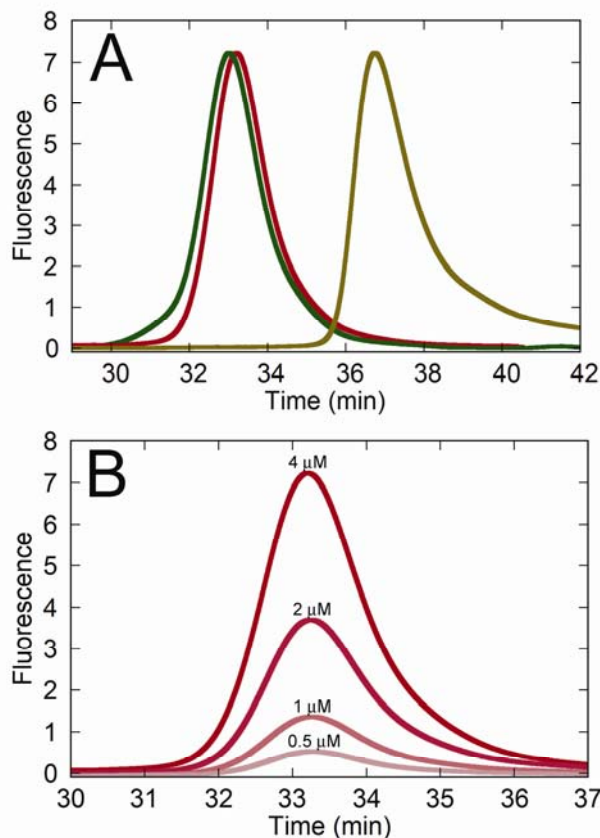
### Individual dimers of the mitotic kinesin motor Eg5 step processively and support substantial loads *in vitro*

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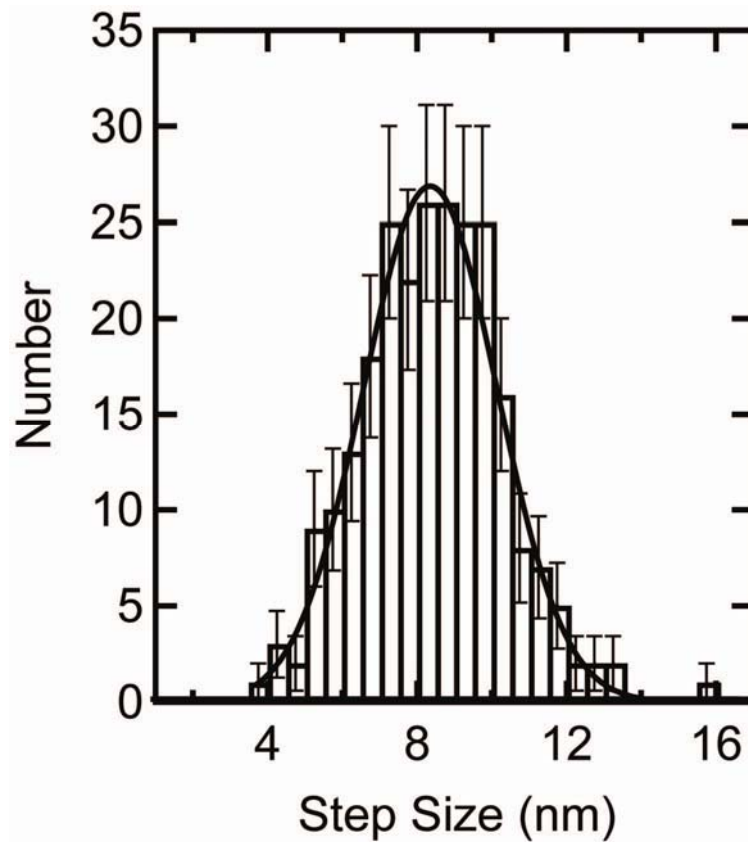
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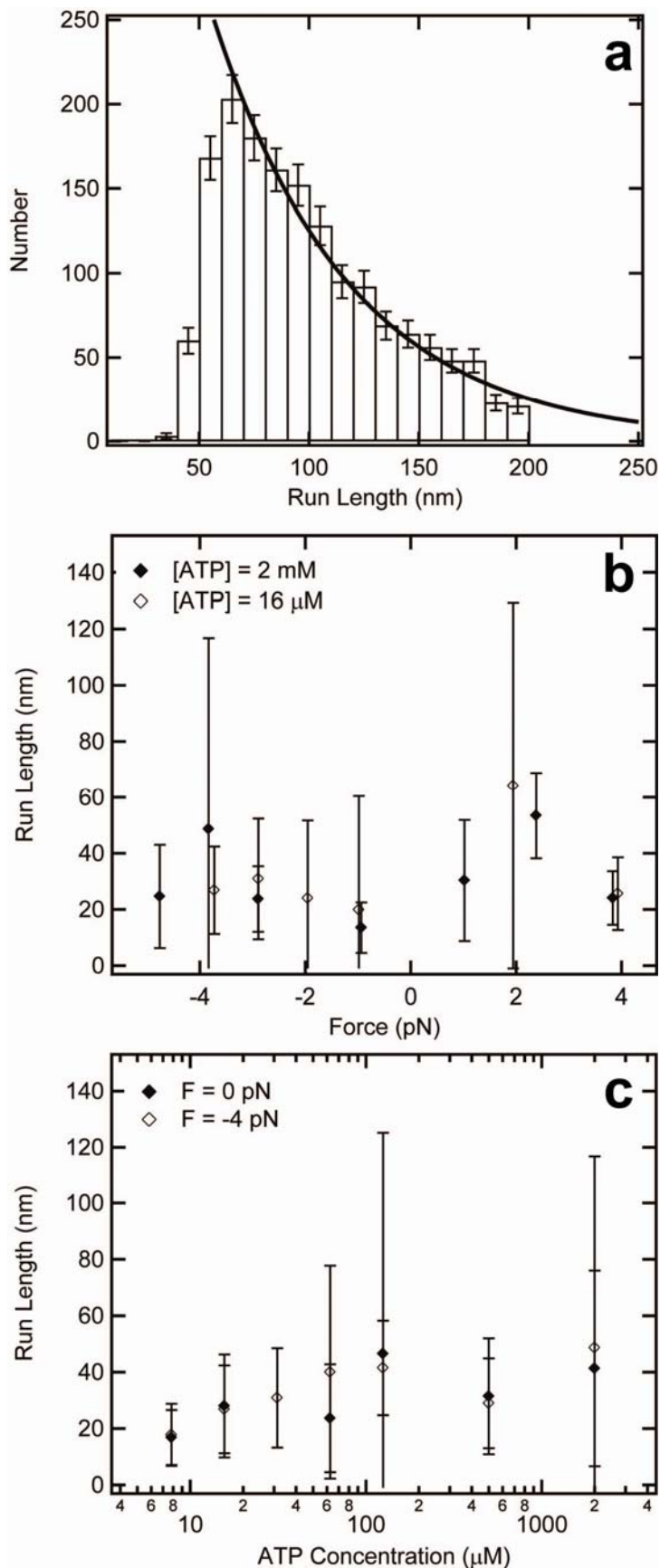
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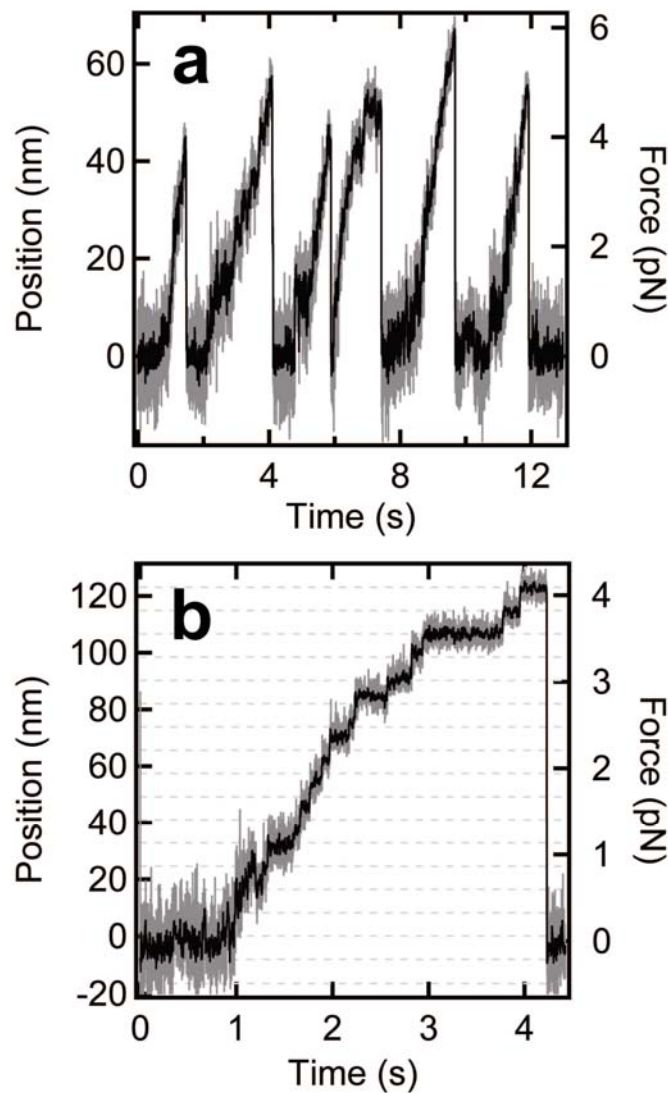
**Figure S1** Analytical gel filtration profiles for Eg5 motors purified from *E. coli*. **(a)** Elution profiles monitoring intrinsic fluorescence vs. time for Eg5-513 (green), Eg5-513-5His (red), and Eg5-367 (brown). The void volume eluted at 16 min and the included volume at 51.5 min. The predicted value for  $M_r$ , based on the amino acid sequence for monomers is 57,644 Da for HsEg5-513, 58,787 Da for HsEg5-513-5His, and 41,667 Da for HsEg5-367. The oligomeric state was determined from plots of the partition coefficient,  $K_{av}$ , vs.  $\log M_r$  (not shown). HsEg5-513:  $M_r = 189,688$  Da; HsEg5-513-5His:  $M_r = 181,036$  Da. **(b)** Gel filtration of HsEg5-513-5His at varying protein concentrations indicates that the dimeric state remains stable at low concentrations.



**Figure S2** Histogram of step sizes for single Eg5-513-5His dimers (bin width, 1 nm;  $N = 257$ ). A Gaussian fit to this distribution yielded a mean step size of  $8.1 \pm 0.1$  nm, a distance consistent with the spacing of tubulin heterodimers along the microtubule protofilament. Note the absence of fractional or multiple step sizes, which can be generated when beads carry multiple motors.



**Figure S3** Run lengths are relatively insensitive to the applied load or to the ATP concentration. **(a)** Global histogram compiled from run lengths for all applied forces and ATP concentrations ( $N = 1,602$ ; min. bin width 10 nm; all bins widths scaled to contain  $>6$  counts). A small number of runs  $>200$  nm exceeded the region reliably monitored by our instrument and were excluded. An exponential fit to this distribution (excluding the first three bins) yielded a mean run length of  $63 \pm 3$  nm, in statistical agreement with the unloaded mean run length determined by video tracking ( $67 \pm 7$  nm)(data in **Fig. 2h**). **(b)** Run length as a function of force at  $[ATP] = 2$  mM (solid diamonds;  $n = 70-212$  for each point) and  $[ATP] = 16$  μM (open diamonds;  $n = 57-80$  for each point), determined by exponential fits to histograms of run length at each load. **(c)** Run length as a function of  $[ATP]$  at  $F = 0$  (solid diamonds,  $n = 22-67$  for each point) and  $F = -4$  pN (open diamonds,  $n = 29-92$  for each point), as determined by exponential fits to histograms of run length at each  $[ATP]$ .



**Figure S4.** Stall force measurements with a fixed-position trap. Single Eg5 dimers step processively from the trap centre under monotonically increasing, hindering loads. **(a, b)** Representative records of bead motion. Median-filtered bead position (15-point window, black trace) is superimposed on the unfiltered position (grey trace). **(a)** Optical trap stiffness,  $k = 0.09$  pN/nm. Beads moved out to  $\sim 65$  nm from the centre of the trap, sustaining loads up to 6 pN. **(b)** Optical trap stiffness,  $k = 0.035$  pN/nm. The bead moved 120 nm from the centre, sustaining a load up to 4 pN, before dissociating. Note that noise decreases at higher loads due to increased stiffness associated with the kinesin-bead linkage. As a consequence, 8-nm-sized steps are more apparent at higher loads.

## SUPPLEMENTARY INFORMATION

### Dependence of $K_M$ on load for Eg5 and conventional kinesin

For kinesin,  $k_b$  decreases more steeply than  $k_{cat}$  with load, causing  $K_M$  to increase<sup>9</sup>. For Eg5, however,  $k_{cat}$  and  $k_b$  appear to vary in equal proportion, making  $K_M$  independent of load. This disparity may be a consequence of differences in nucleotide affinity between the two motors. For kinesin, the  $K_D$  for nucleotide binding is relatively high ( $K_D = k_{-1}/k_1 \approx 400 \mu\text{M}$ ) as compared with the unloaded  $K_M$  ( $\sim 80 \mu\text{M}$ )<sup>9</sup>; therefore, the application of force can slow steps that occur after binding until  $K_M$  approaches  $K_D$ . In contrast, the unloaded  $K_M$  for Eg5 is just  $14 \mu\text{M}$ , and fits to the three-state model (presented in the main text; see Fig. 3c) return a comparable value for  $K_D$ , leaving little ‘headroom’ for  $K_M$  to increase with force.