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Supporting Material

Force and premature binding of ADP can regulate the processivity of individual Eg5 dimers

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SUPPLEMENTARY MATERIALS FOR:

Force and premature binding of ADP can regulate the processivity of individual Eg5 dimers

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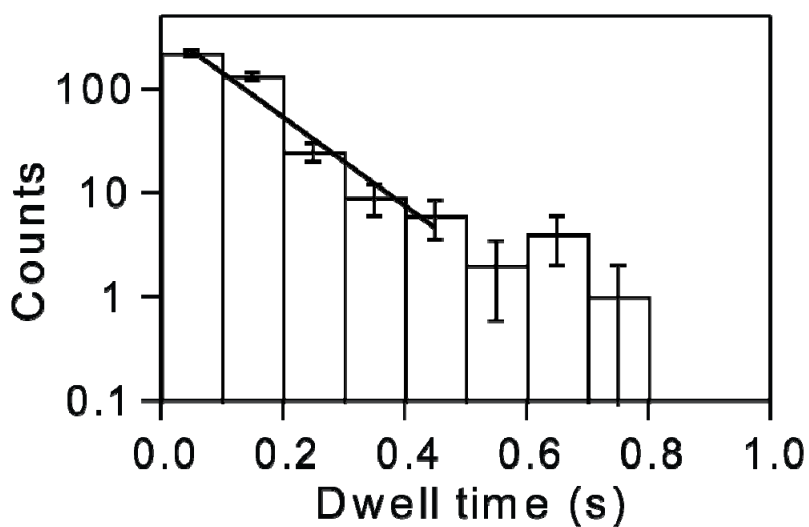


Figure S1. Representative dwell time distribution for 2 mM ATP, 10 mM P_i and $F = -3.0 \pm 0.1$ pN. The exponential line-fit (solid line) corresponds to a dwell time of 0.10 ± 0.06 s (mean \pm s.e.). The arithmetic mean of this distribution is 0.13 ± 0.01 s (mean \pm s.e.).

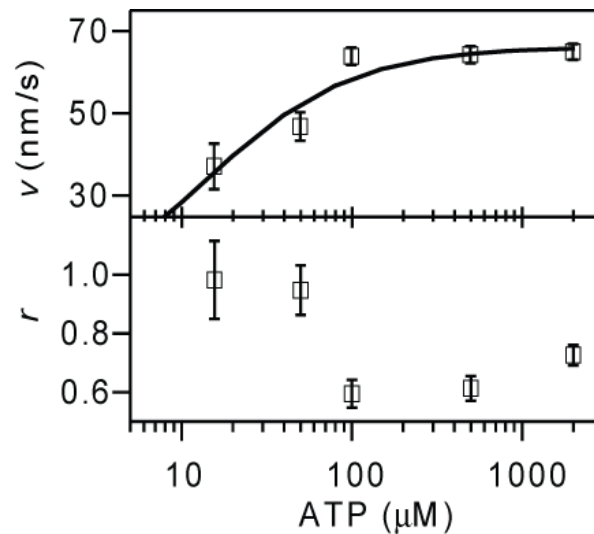


Figure S2. Velocity and randomness as functions of ATP concentration for $F = -3.0 \pm 0.1$ pN (mean \pm std. dev., $N = 17$ -209 runs at each condition). Velocity, v , showed a Michaelis-Menten dependence on ATP concentration (upper panel). The solid line is the expected curve based on a three-state mechanochemical model with a reversible ATP binding transition followed by an irreversible force-dependent translocation and an irreversible biochemical transition to end the cycle, using previously published rate constants for Eg5-513-His (Valentine, *et al.*, 2006). Randomness data, r , showed the same dependence on the ATP concentration as previously measured (lower panel).

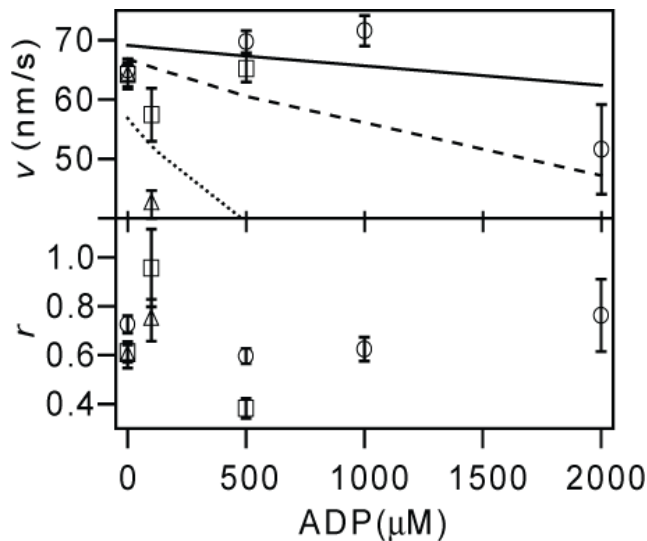


Figure S3. Velocity and randomness as functions of the ADP concentration for $F = -3.0 \pm 0.1$ pN and ATP = 2000 μ M (circles, $N = 8$ -207; solid line), 500 μ M (squares, $N = 22$ -115; dashed line), 100 μ M (triangles, $N = 33$ -100; dotted line). Lines are global fits to a competitive inhibition model with $v = v_{\max} [ATP] / ([ATP] + K_M(1 + [ADP]/K_I))$; v_{\max} is the maximal velocity, K_M is the apparent Michaelis constant for ATP binding, and K_I^{ADP} is an ADP-dependent inhibition constant. We found $v_{\max} = 70 \pm 1$ nm/s, $K_M = 23 \pm 5$ μ M, in good agreement with ATP- and phosphate-dependent data and with $K_I^{ADP} = 210 \pm 90$ μ M (mean \pm s.e.).

Valentine, M. T., P. M. Fordyce, T. C. Krzysiak, S. P. Gilbert, and S. M. Block. 2006. Individual dimers of the mitotic kinesin motor Eg5 step processively and support substantial loads *in vitro*. *Nat. Cell Biol.* **8**: 470-476.