Supporting Text

Correction for Concatenation of Pauses. There are two cases where adjacent pauses may incorrectly be interpreted as a single pause. In the first case, BeF_x release from the front head is followed by binding of BeF_x to the rear head without any intervening forward steps. The new extended dwell induced by binding of the second BeF_x molecule will then immediately follow the terminal backstep dwell associated with the original BeF_x molecule. As a result, one terminal backstep will be missed. In the second case, the release and binding of BeF_x are separated by a single forward step, causing the terminal backstep to be mistaken for a recurrent backstep.

   To adjust the data for the effects of these comparatively rare events, a correction factor was applied to the mean recurrent backstep dwell time and R_b. Taking the probability of pausing to be P_p and the probability of stepping to be $P_s = 1 - P_p$, then the chance of the first case of concatenation occurring within any pause will be given by P_p. The second case occurs with probability $P_p(1 - P_p)$. The value of P_p was estimated by forming the ratio of observed pauses to net forward steps, f_p', related by the expression

$$P_p = \frac{f_P'}{\left(1 - f_P'\right)},$$ [1]

where f_p' was computed from the ratio of all pauses observed to the total distance advanced between pauses, divided by the step size (which equals the numerical difference in the number of forward and backward steps). A corrected value for the pause frequency, f_p, can by computed from the ratio P_p/P_s, where P_p is the pause probability computed from Eq. **1** and P_s is the net stepping probability.

Given an estimate of P_p, the corrected number of terminal backsteps can be obtained from

$$N_{tb} = \frac{N'_{tb}}{(1 - P_p)^2},$$ [2]

where N_{tb} is the corrected number of terminal backsteps and N_{tb}' is the measured number of terminal backsteps. Similarly, the corrected number of recurrent backsteps can be obtained through

$$N_{rb} = N'_{rb} - N_{tb}\left[P_p(1 - P_p)\right],$$ [3]

where N_{rb} is the corrected number of recurrent backsteps and N_{rb}' is the measured number of recurrent backsteps. The ratio of N_{rb} to N_{tb} was used to compute the corrected value for R_b. The mean recurrent backstep dwell time can also be corrected by using the estimate of P_p through

$$\tau_{rb} = \frac{\tau'_{rb}\left(N_{rb} + N_{tb}\left[P_p(1 - P_p)\right]\right) - N_{tb}\tau'_{rb}\left[P_p(1 - P_p)\right]}{N_{rb}},$$ [4]

where τ_{rb} is the corrected mean recurrent backstep dwell time, τ_{rb}' is the measured mean recurrent backstep dwell time, and τ_{tb}' is the measured or actual mean terminal backstep dwell time (because the average length of this interval is unaffected by any concatenation of pauses).

Multiple Kinesin States During Processive Stepping Are Competent to Bind BeF$_x$.
Increasing the proportion of the stepping cycle (Fig. 1) occupied by any state where kinesin is competent to bind BeF$_x$ should result in an increase in the pause frequency, f_p. Prolonging the duration of either states F1 or R2 by lowering [ATP], or states F2 or R1 by increasing the load, is expected to increase the proportion of time during which an ADP-bound head is available for binding BeF$_x$. Consistent with this prediction, we found that f_p increased when load was increased or when ATP concentration was reduced (Fig. 5). We conclude, therefore, that a

significant fraction of BeF_x binding occurs during states F1 and F2 (or during R1 and R2). However, we were unable to exclude other states (such as F3, R3, or R4) as candidates where significant BeF_x binding occurs. The uncertainty in f_p was estimated by assuming that the fractions of both pauses and net forward steps were binomially distributed. The binomial error, computed as in ref. 1, was added in quadrature to an error term for instrument drift in the calculation of N_{steps}. Statistical analysis revealed that at low load, $\approx$5-10 % of pauses may have been missed because of noise (below). Correction of the data for these missing events, which was not performed, would result in a negligible change to the load dependence of the pause frequency (Fig. 5).

Choice of BeF_x over AMP-PNP. Pauses induced by BeF_x binding occurred more frequently at high loads (Fig. 5), in contrast to pauses induced by AMP-PNP, which occurred with a frequency more or less independent of load (data not shown). As a consequence, in the presence of AMP-PNP, kinesin generally entered a long pause at low load soon after encountering the MT, before the force clamp could be activated. Data could therefore only be usefully collected after waiting for this lengthy pause to terminate, hampering the collection of large numbers of pauses because of time constraints. In contrast, long pauses induced by BeF_x generally occurred after the activation of the force clamp (which raised the load), facilitating more rapid accumulation of statistics. The difference in pause frequency between the two analogs arises because AMP-PNP binds to a nucleotide-free head, whereas BeF_x binds to an ADP-bound head. Because the relative proportions of the kinesin stepping cycle populated by the nucleotide-free and ADP-bound states differ (Fig. 1), one expects that the binding kinetics of the two analogs will differ as a function of load, exactly as observed.

Missing and False Events. To assess the accuracy of the event detection algorithm, simulated data were generated by superimposing 8-nm backsteps of fixed duration on a trace of baseline noise. The noise was simulated by averaging the Fourier transform from several extended dwell regions obtained experimentally, then performing the inverse transform with random phasing. Above the temporal cutoff (selected to be 1 ms less than the width of the time window of the median filter applied to the data), false events constituted ≤2% of the total number of events detected, and there were no missing events. To account for missing events below the cutoff, we applied a correction factor that assumes dwell times remain nearly exponentially distributed (2). For recurrent and terminal backstep dwells, the mean dwell time was corrected by using

$$\tau_c = \tau - \xi,$$ **[5]**

where τ_c is the corrected mean backstep dwell time, τ is apparent mean of backstep dwell times above the resolution cutoff, and ξ represents the cutoff value. Extended dwells, which become concatenated when recurrent backsteps are missed, can also be corrected. In this case, the corrected mean extended dwell time is given by

$$\lambda_c = (\tau_{rb} + \lambda)\exp(-\xi/\tau_{rb}) - \lambda,$$ **[6]**

where λ_c is the corrected mean extended dwell time, and λ is the apparent mean extended dwell time. We also used the estimate of missed recurrent and terminal backsteps to correct R_b. In all cases, errors were propagated by standard methods after corrections were applied.

Dwell Time Analysis. Approximately 10% of pauses were prematurely terminated by kinesin dissociating from the MT. In such cases, the backstep and extended dwells immediately before

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dissociation were not selected for analysis. The distributions of extended dwell intervals, recurrent backsteps, and terminal backsteps are shown before missing event correction (Fig. 6).

References

1.   Svoboda, K. & Block, S. M. (1994) *Cell* **77,** 773-784.

2.   Colquhoun, D. & Hawkes, A. G. (1995) in *Single-Channel Recording*, eds. Sakmann, B. & Neher, E. (Plenum, New York), pp. 397-482.