Supplementary Material (ESI) for PCCP

Supplementary methods

Cloning and protein preparation

Our fast kinesin motor is a *Drosophila* kinesin construct containing residues 1-576 followed by a carboxy-terminal 6x-histidine tag. This construct was created using PCR mutagenesis from a full-length *Drosophila* kinesin construct that was the gracious gift of D.D. Hackney (Carnegie Mellon University). The five point mutations in the slow motor were introduced into the same constuct according to Stratagene's Quikchange mutagenesis protocol using the following primers:

5'-CAGGAGTCGCTGGCGGCGAACGCACGCAC-3'

5'-GTGCGTGCGTTCGCCGCCAGCGACTCCTG-3'

5'-CGTAGAGCCAAGACAGCAGCTGCCGTGGTCTGCGTTAACG-3'

5'-CGTTAACGCAGACCACGGCAGCTGCTGTCTTGGCTCTAC-3'.

Sequences of fast and slow kinesin were verified after mutagenesis. *E. Coli* BL21-DE3 cells were transformed with the construct in fresh pET17b expression vector, grown for 8 hours, and induced with 0.1mM IPTG overnight. Cells were lysed by french press in lysis buffer (50 mM HEPES (pH 7), 500 mM KCl, 20 mM imidazole, 4mM $MgCl_2$,1 mM EGTA, 10μM ATP, 5 mM β-mercaptoethanol, leupeptin (1 μg/ml), pepstatin (1 μg/ml), aprotinin (1 μg/ml)), PMSF (10μg/ml). Supernatants were incubated with Ni-NTA resin (Qiagen) for 2 hours and applied to a disposable column. The resin was washed with with 200mM imidazole in lysis buffer and eluted with a final concentration of 500mM imidazole. Peak fractions were frozen (5% sucrose added) and stored in liquid nitrogen. Concentrations of kinesins were determined using an SDS-PAGE gel with BSA standards.

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Comparison of Alternating Action and Mechanical Competition models for determining the relationship between $\frac{n_f}{n}$ and velocity.

Two methods are described below for estimating the number of fast and slow motors bound to a cargo moving at a specific velocity. Both are modified from Pan, et al. [1].

Following the Pan, et al. alternating action model, the velocity of a microtubule V_{mt} which is being moved by n non-cooperative, non-interacting motors can be described by equation (S1).

(S1) $$V_{mt} = \sum_{n} \frac{Distance_n}{Time_n}$$

Because both types of motors take 8-nm steps on microtubules in our system, this is equivalent to equation (S2), where n_s is the number of slow motors and n_f is the number of fast motors moving the microtubule, V_s is the slow motor velocity, and V_f is the fast motor velocity.

(S2) $$V_{mt} = \frac{8\ nm * (n)}{\frac{8\ nm\ *\ n_f}{V_f} + \frac{8\ nm\ *\ n_s}{V_s}}$$

Rearranging equation (S2) and defining $n_s + n_f = n$, Pan, et al. obtained

(S3) $$V_{mt} = \frac{n * V_s * V_f}{n_f * V_s + n_s * V_f}$$

We can write this in terms of $\frac{n_f}{n}$ as follows:

(S4) $$\frac{n_f}{n} = \frac{V_f * (V_{mt} - V_s)}{V_{mt} * (V_f - V_s)}$$

This is the same as equation (1) from the text.

Pan, et al. also described a mechanical competition model, in which the forces exerted by the two motors on each other balance. These forces are written:

(S5) $$F_+ = \left(1 - \frac{V_{mt}}{V_f}\right) n_f F_{stall} \qquad F_- = \left(1 - \frac{V_{mt}}{V_s}\right) n_s F_{stall}$$

This is identical to eq. (2) in the text. Because $F_+ = -F_-$ when the forces balance,

(S6) $$\frac{V_{mt}}{V_f} n_f + \frac{V_{mt}}{V_s} n_s = n_s + n_f$$

(S7)

$$V_{mt} = \frac{(n_s + n_f) V_s * V_f}{n_s * V_f + n_f * V_s}$$

Rearranging in terms of $\frac{n_f}{n}$, we obtain eq. (S8), which is equivalent to eq. (5) in the text.

(S8) $$V_{mt} = \frac{V_s * V_f}{\left(1 - \frac{n_f}{n}\right) * V_f + \left(\frac{n_f}{n}\right) * V_s}$$

$\frac{n_f}{n}$ can then be written in terms of the velocities:

(S9) $$\frac{n_f}{n} = \frac{V_s * V_f - V_f * V_{mt}}{V_s * V_{mt} - V_f * V_{mt}}$$

(S10) $$\frac{n_f}{n} = \frac{V_f * \left(V_{mt} - V_s\right)}{V_{mt} * \left(V_f - V_s\right)}$$

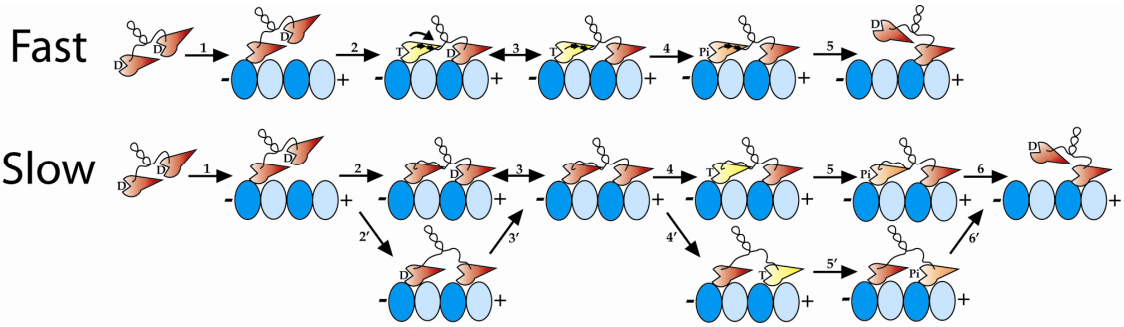
Eq. (S10) is equivalent to eq. (S4) and eq. (1) in the text. Therefore, both the alternative action and mechanical competition models described by Pan, et al. [1] can be used to obtain the same expression for $\frac{n_f}{n}$ in terms of velocities, provided that the stall forces of the two motors in question are the same.

Supplementary figure 1:

Possible kinetic models of fast and slow kinesin movement. This figure was modified from [2]. Fast kinesin is believed to follow the scheme shown on the top. One head releases ADP upon binding to microtubules (step 1). ATP binding induces neck linker zipping, biasing the unbound head forward (step 2). The unbound head binds weakly in front and releases ADP (step 3). When the front head binds tightly to the microtubule (step 4), phosphate release by the rear head proceeds rapidly (step 5). At that point, the motor is in the same configuration as step 2, having taken a single step toward the MT plus end. This scheme essentially follows that described in [3]. Slow kinesin is hypothesized to follow the set of schemes shown on the bottom. One head releases ADP upon binding to microtubules (step 1), as for fast kinesin. Neck linker docking is deficient, leading to slow ATP binding by the MT-bound head [4]. Therefore, the unbound second head can release ADP and bind to the microtubule before the first head binds ATP. The second head therefore binds either forward or rearward with very little bias (step 2 or 2'). Release of ADP by the second head can lead to an apo/apo state (step 3). ATP binding and hydrolysis can then occur in either head (steps 4 and 5 or 4' and 5'), followed by phosphate release (step 6 or 6'). Again the motor is in the same configuration as step 2, having taken a forward step (1-2-3-4-5-6), a backward step (1-2'-3'-4'-5'-6'), or no step (1-2'-3'4-5-6 or 1-2-3-4'-5'-6').

Fast and slow kinesins are likely to spend virtually identical amounts of time in the slower steps of this cycle, because the two motors have identical Kcat values (**Figure 1C**). The difference is that slow kinesin can transiently go into an Apo/Apo state (between steps 3-4) that is very tightly bound to microtubules. Hence, the two motors

have identical Kcat values, but slow kinesin has a lower Km(MT) and a much slower velocity **(Figure 1C)**.



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