External mechanical force as an inhibition process in kinesin's motion

Aleix CIUDAD[1] and José María SANCHO

Departament d'Estructura i Constituents de la Matèria, Facultat de Física, Universitat de Barcelona, Diagonal 647, 08028, Barcelona, Spain

We analysed published force–velocity data for kinesin using classical Michaelis–Menten kinetic theory and found that the effect of force on the stepping rate of kinesin is analogous to the effect of a mixed inhibitor in classical inhibition theory. We derived an analytical expression for the velocity of kinesin (the stepping rate, equal to the ATP turnover rate) as a function of ATP concentration and force, and showed that it accurately predicts the observed single molecule stepping rate of kinesin under a variety of conditions.

Key words: kinesin dynamics, inhibition process, load dependence, mechanical force, molecular motor.

INTRODUCTION

The kinesin superfamily is a set of proteins that are involved in the mechanisms of transport inside eukaryotic cells. Members of this family are responsible for carrying organelles such as vesicles or mitochondria along microtubules, which are the rigid structures that define the axial direction of the transport. Conventional kinesin is a processive two-headed and ATP-driven molecular motor that moves along tubulin subunits with discrete steps of approx. $l_0 = 8$ nm, alternating the heads in each step. Recent single-molecule experiments using optical tweezers have revealed two important features. First, the 8-nm stepping is one-to-one coupled with ATP hydrolysis [1]. This means that the free energy source of an ATP molecule is needed to perform a single step. This mechano-chemical coupling will allow us to relate kinetics to mechanical forces. The second important feature is the variation of the mean velocity as a function of ATP concentration, [ATP], and an axial external force opposite to the movement [2]. Upon increasing the force, the velocity decreases until it becomes zero at a maximum value called stall force, which is typically approx. 5–7 pN. On the other hand, when [ATP] is modified at constant F, the velocity behaves in a Michaelian way, so the corresponding Michaelis–Menten analysis can be applied.

The kinetic cycle of the two heads of the dimer is related to the mechanical cycle in such a way that one head performs the step when it has an ADP bound in the catalytic site, while the other remains free of nucleotide and is attached strongly to the microtubule. When the first head attaches to the next tubulin unit, the second head is then allowed to bind an ATP molecular and hydrolyse it. We cannot understand the chemical cycle without taking into account the co-operation of the two heads.

Our main goal is to adjust an analytical expression for the velocity of the motor as a function of these two relevant variables, F and [ATP], using only four parameters. In order to achieve it, we will consider the force to be an inhibitor of the enzymatic activity. Then, using the available experimental data [2] and standard criteria [3,4], we will see that the force can be adequately modelled as a reversible and full mixed-competitive inhibitor. This classification will allow us to discuss the force sensitivity of each of the two chemical states. The next step will consist of determining the relation between the value of the applied force and the equivalent effective inhibitor concentration, $[I]_F$. Later, taking into account the mechano-chemical coupling, we will obtain an analytical expression for v([ATP],F) that can be compared with the experimental data. It is worth commenting that factors such as ionic strength, pH and other details of the experimental setup can modify slightly the values of some parameters, but are accommodated readily by the model, so, under different conditions, the fitting process can be redone to recalibrate them. We will also discuss how this analysis can be extended to loads that assist movement without changing the formalism.

Finally, as a collateral result, we extend our analysis beyond first-order statistics with a study of the randomness, which is a parameter that sheds light on the more subtle properties of kinesin's dynamics, such as the coupling ratio and the number of rate-limiting steps.

The behaviour of kinesin has been the subject of a considerable amount of theoretical work [5–7], but no model has been able to reproduce the experimental data with high accuracy without using a large set of adjustable parameters. We believe that, owing to its simplicity and precision, the kinetic description of the present paper constitutes an attractive approach to study the kinesin's motion.

EXPERIMENTAL

In the last decade of the twentieth century, nanotechnology revolutionized the way of approaching the cell's biology. Fibre optics, optical tweezers and other devices have allowed us to study the properties of single molecules. In particular, several groups have conducted a series of experiments in order to study single conventional kinesins walking along microtubules using optical traps [1,2,8–11]. This laser-based mechanism puts the protein under a harmonic potential of stiffness 0.037 pN/nm. Then, after attaching it to a silica bead 0.5 μm in diameter, the displacement of the kinesin under that potential can be translated into the corresponding force using Hook's law. On the one hand, the kinesin is allowed to run away until it reaches its maximum force. On the other hand, it can also follow a constant-force trajectory using a feedback system that moves the centre of the trap maintaining constant separation from the kinesin. In 1999, Visscher et al. [2] published a series of results on the kinesin's v against [ATP] and the external force, F [2]. These results are the inputs of our study. As mentioned above, the kinetics of the free-inhibitor chemical processes involved in the kinesin cycle were described by the so-called Michaelis–Menten law, eqn 1,

$$v = V_{\max}\frac{[\mathrm{ATP}]}{K_{\mathrm{m}} + [\mathrm{ATP}]} \quad (1)$$

[1] To whom correspondence should be addressed (email aciudad@ecm.ub.es).

Table 1 Kinetic parameters for different forces from [2]

F (pN)	V_{max} (nm/s)	K_m (μM)
1.05	813 ± 28	88 ± 7
3.59	715 ± 19	140 ± 6
5.63	404 ± 32	312 ± 49

where V_{max} is the velocity at saturated [ATP], and K_m is the concentration at which the velocity is half that of V_{max}, i.e. S_{50}. These two kinetic parameters can be adjusted from experimental data, leading to the values shown in Table 1. One can see how V_{max} decreases while both K_m and F increase. Although the value of V_{max} was expected to decrease for increasing force levels, the force-dependence of K_m was an unexpected discovery. This scenario has motivated us to relate the force with inhibition theory, where non-constant values of V_{max} and K_m are considered. Thus we will not follow the standard approach of including the mechanical work done by the external force as an additive term in the free energy potential [12].

THEORY

Inhibitors

An inhibitor is, commonly, a substance that reduces the velocity of a reaction catalysed by an enzyme. The mechanisms involved are basically attachments that forbid or make difficult the binding of the substrate. However, because inhibition theory is purely kinetic, we can conjecture that other factors, such as a mechanical force, can also be considered as an inhibitor if they produce similar kinetic consequences. In such a scenario, we first have to classify to what type of inhibition the mechanical force belongs [3,4]. There are two main groups of inhibitors: irreversible and reversible. Because kinesins recover their V_{max} when we stop applying the load, we can consider that the force belongs to the reversible group. Then, a reversible inhibitor can be partial or full, depending on the remaining velocity that they allow to work to the enzyme at high inhibitor concentration. Of course, the force is able to cancel the directional movement (when it reaches the stall force value), so it can be considered as a full inhibitor. Finally, a reversible full inhibitor can be of four different types depending on how it affects to the Michaelian parameters V_{max} and K_m when inhibitor concentration, $[I]_F$, grows. This specific class can be determined from the experimental values shown in Table 1. It should be accepted that an increasing force can be interpreted as an increasing $[I]_F$, so we can see how V_{max} decreases and K_m grows, and therefore the ratio V_{max}/K_m decreases as we increase the value of $[I]_F$. With all this information, the classification can be completed. The external mechanical force acts as a mixed competitive inhibitor [3,4]: mixed because both Michaelian parameters change, and competitive because the ratio V_{max}/K_m decreases. In order to test quantitatively this classification, we can use the standard graphical criteria that appear in the literature. In Figure 1, we plot the experimental data from [2] against [ATP]. Downward (V_{max} decreasing)-rightward (K_m increasing) arrows indicate the standard type of inhibition. There are several ways to classify inhibitors. Specifically, pharmacologists characterize them using $-i_{0.5}$, the inhibitor concentration at which the reaction rate becomes infinite [13]. The usual scenario for mixed inhibition is what we show in Scheme 1, which is the chemical cycle for one head of the dimer. K_{ic} is the competitive constant, and K_{iu} is the uncompetitive constant. Because the inhibition is mixed

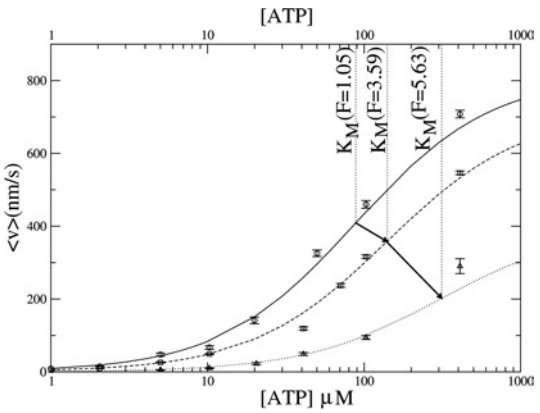
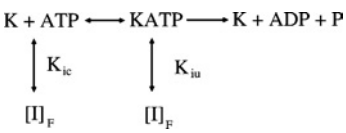


Figure 1 Mean velocity against [ATP] for three external forces

The vertical axis is a linear scale to visualize better the different Michaelis constants. For all the Figures, points are taken from [2] and lines are theoretical predictions. Arrows are guides for classification.



Scheme 1 Mixed inhibition of the force represented by $[I]_F$

competitive, $K_{iu} > K_{ic}$, both of them being dissociation constants, (the greater its value, the weaker the corresponding inhibition). The determination of these two constants will give us an index of force-sensitivity of each state. K_{iu}^{-1} indicates how much the force affects the ATP-bound state, or, in other words, how much the load reduces the rate of ATP hydrolysis. K_{ic}^{-1} measures the sensitivity of kinesin's ATP-free state to the force, so it determines the rate at which one head of the motor performs the power stroke and then the other head binds an ATP from the cell medium.

Mechanical inhibition

In this section, we will define a quantitative relationship between the mechanical force, F, and its equivalent inhibitor effective concentration, $[I]_F$. When $F = 0$, the velocity is given by the Michaelis–Menten law (eqn 1). However, when an inhibitor is acting upon the enzyme, this law is no longer valid. Taking into account the scenario of Scheme 1, we see that two more parameters, the inhibition dissociation constants K_{iu} and K_{ic}, are required to complete the equation for the velocity. Although this fact will be translated into a more complicated expression, a convenient notation can be introduced for the sake of clarity, allowing us to keep the essence of the original Michaelis–Menten law. The resulting modified Michaelis–Menten law becomes:

$$v = V_{max}(I)\frac{[\mathrm{ATP}]}{K_m(I) + [\mathrm{ATP}]} \tag{2}$$

where $V_{max}(I)$ and $K_m(I)$ are the apparent Michaelian parameters, each one related with its correspondent real (free force) Michaelian parameter and the inhibition constants by

$$V_{max}(I) = \frac{V_{max}}{1 + \dfrac{[I]_F}{K_{iu}}} \tag{3}$$

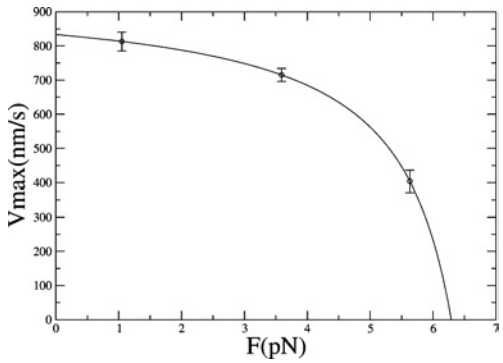


Figure 2 Fit of V_{max} as a function of F according to eqn (3)

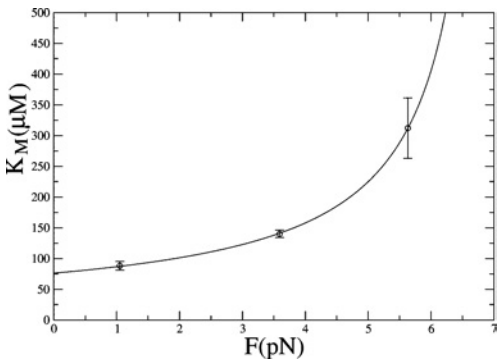


Figure 3 Fit of K_m as a function of F according to eqn (4)

and

$$K_m(I) = K_m \frac{1 + \frac{[I]_F}{K_{ic}}}{1 + \frac{[I]_F}{K_{iu}}} \quad (4)$$

In order to relate these formulae [3] with the mechanical force, it is worth recalling the concept of stall force F_S. From the experiments, we know how the kinesin motor can still work against the load up to a maximum value of F_S, which can be interpreted as the force that the motor extracts from ATP hydrolysis or maybe the strength of the microtubule's binding. The ratio of both forces, F_S/F, becomes an adimensional quantity that gives us a measure of the force's balance. However, what we want is that the free force case, $F = 0$, which corresponds to a null concentration of inhibitor, $[I]_F = 0$, and that $F = F_S$, the maximum force case, corresponds to $[I]_F \to \infty$. This is what one gets when expressing the dependence of $[I]_F$ on F in the following simple way:

$$[I]_F = \left(\frac{F_S}{F} - 1\right)^{-1} \quad (5)$$

Substituting eqns (3) and (4) into eqn (2), we obtain the velocity of the kinesin, $v([ATP],[I]_F)$, as a function of the two control variables:

$$v = V_{max}[ATP]\left\{K_m\left(1 + \frac{[I]_F}{K_{ic}}\right) + [ATP]\left(1 + \frac{[I]_F}{K_{iu}}\right)\right\}^{-1} \quad (6)$$

The force dependence is shown implicitly for more clarity. To obtain the full expression, it is enough to substitute eqn (5) into eqn (6). This equation is our main result. It constitutes an analytical expression for the kinesin velocity as a function of [ATP] and an external and axial mechanical force. It has only four parameters, the two inhibition constants and the usual Michaelian parameters, so its relevance may be judged by considering the good relation between accuracy and the number of adjustable coefficients.

RESULTS

We only need to use the values from Table 1 in order to get the four free parameters. More precisely, from the expression of $V_{max}(I)$ (eqn 3) and from a value of $F_S = 6.3$ pN we fitted V_{max} and K_{iu} (see Figure 2), and from the expression $K_m(I)$ (eqn 4), we complete the fit, making use of the values of K_m and K_{ic}. (see Figure 3). The results obtained are shown in Table 2, and

Table 2 Values of the fitted parameters

Parameter	Value
V_{max} (nm/s)	833.26
K_m (μM)	76.1
K_{ic} (μM)	1.15
K_{iu} (μM)	8.04

it is worth commenting on them. First, we have estimated that the saturated velocity at null force is $v = 833$ nm/s. This is the maximum speed that kinesin can reach under a saturated [ATP], in agreement with the extrapolated data. The Michaelis constant is 76.1 μM, which indicates to us that, in the absence of an external force, $[ATP] = 76.1$ μM is the 50 % saturating [ATP], S_{50}. As an example, the motor will reach 90 % of its V_{max} at $[ATP] = 9K_m \approx 685$ μM. However, the most interesting part of the analysis focuses on the inhibition constants. As shown in Scheme 1, when the motor is free from the ATP nucleotide, the functional inhibition constant is $K_{ic} = 1.15$ μM. Then, when an ATP molecule binds to the protein, the inhibition constant changes to $K_{iu} = 8.04$ μM ($K_{ic} < K_{iu}$, according to the competitive subclass). As these are dissociation constants, the ATP hydrolysis process is less sensitive to F than the rest of the kinetic cycle. One can think that a mechanical pulling force should not affect a binding rate that is controlled mostly by temperature, but one has take into account that one head of the dimer will not bind an ATP until the other has finished the previous step. Then, because of the co-ordination between the two heads, the K_{ic} affecting rate encloses the power stroke phase of the previous step and the diffusive binding time of the next cycle. Maybe the diffusive time scale is not affected by F, but it is clear that the pulling load will have its maximum effect while one head is detached from the microtubule and performs the step. It has to be admitted that the real mechano-chemical cycle of kinesin is much more complex than the two-state model of the present paper, but it is also evident that such a simplification permits quite accurate adjustments. Now, we compare our predictions with the experimental data of [2]. In Figure 4, we present the theoretical predictions and experimental data for v against [ATP] for three different constant forces. We can also plot the v against the force F for two different constant [ATP]s (see Figure 5). Considering that Figures 4 and 5 are both predicted from eqn (6), the agreement is quite appealing. Even the concavity of v against F curve at high [ATP] is predicted.

In a more recent experiment [14], a two-dimensional optical force clamp was used to test the response of the kinesin under lateral and assisting loads. It was found, in contrast with the

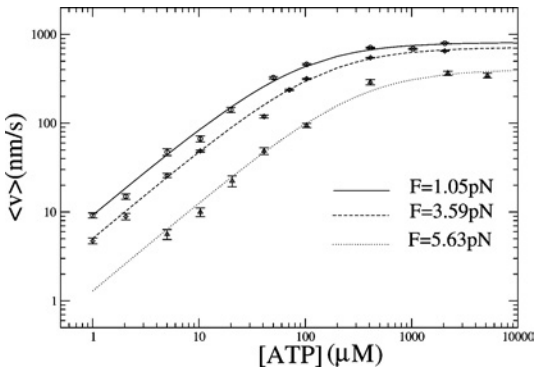


Figure 4 Mean velocity against [ATP] in a log–log plot as is usual in motor literature

In this and the next Figures, lines are our theoretical predictions, i.e. plots of eqn (6).

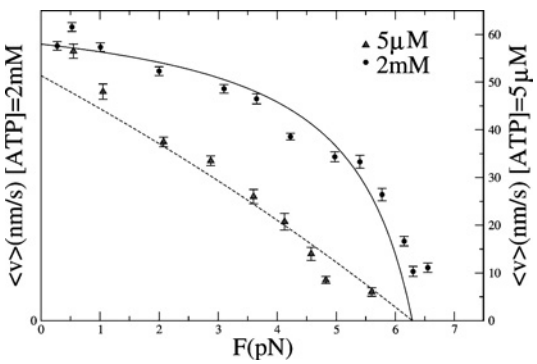


Figure 5 Mean velocity against *F* for [ATP] = 5 and 2000 μM

Forces are opposite to the movement, although here they are taken as positive.

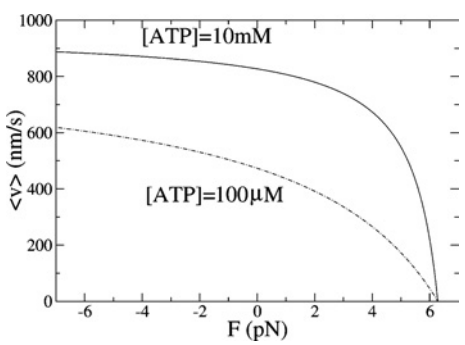


Figure 6 Mean velocity against *F* for different [ATP]

Assisting loads are taken as negative.

results of [10], that when the kinesin was pulled in the sense of the movement (that is, assisting it), the mean velocity did not increase dramatically. The motor was also pulled perpendicularly to the microtubule's symmetry axis. In our model, we cannot incorporate a sideways force without increasing the number of degrees of freedom. However, the assisting load can be considered without doing any change to the formulae. One only needs to change the sign of F and to consider the inhibitor to be negative, that is, an enhancer of the rates involved. In Figure 6, we can see the prediction of eqn (6) (with the parameters of Table 2) for both assisting and opposing loads having different [ATP] levels. We

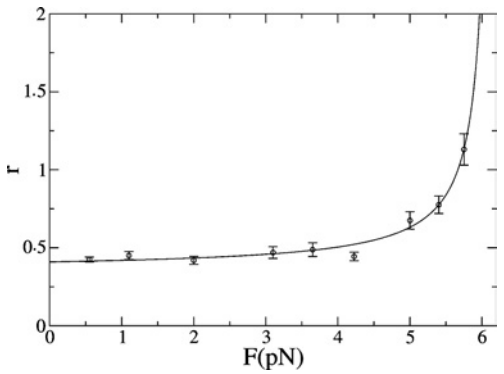


Figure 7 Randomness parameter against *F* for [ATP] = 2 mM

can see that, in our equations, an assisting load increases the velocity, but not significantly, in qualitative agreement with the experiment mentioned.

In order to complete our analysis, we have attempted to fit the experimentally measured randomness, defined as

$$r = \lim_{t\to\infty} \frac{\langle \Delta x(t)^2 \rangle}{l_0 \langle x(t) \rangle} \tag{7}$$

This is a statistical second-order magnitude, proportional to the second moment. If one considers that this second moment represents a diffusion process, it can be approximated by

$$\langle \Delta x(t)^2 \rangle \approx 2Dt$$

(where D is the diffusion coefficient). As one can write

$$\langle x(t) \rangle \approx \langle v(t) \rangle t$$

then,

$$r \approx \frac{2D}{l_0 \langle v(t) \rangle} \tag{8}$$

Let us assume that the diffusion coefficient D does depend mainly on the temperature. Adjusting its value to $D \approx 1350\ \mathrm{nm}^2/\mathrm{s}$ for [ATP] = 2 mM, we obtain the prediction shown in Figure 7. However, at low [ATP], the previous assumptions do not necessarily have to be true, and the predictions do not fit well with experimental data, although we obtain higher randomness for lower [ATP], as expected. The inverse of the randomness parameter provides an idea of how many rate-limiting states are involved in the process. At high [ATP] and low loads, the randomness is approx. 0.4, which indicates that there are approx. three rate-limiting states (maybe diffusive ATP-binding, hydrolysis and phosphate-releasing), while, for high opposing forces and low [ATP], r increases, showing that a single factor becomes the only limiting rate (load and ATP-binding respectively). The r parameter is also related with the mechano-chemical coupling, that is the relation between the full cycle's rate and the physical velocity. As long as the randomness is constant for different loads, the coupling ratio also remains constant. In [2], the value of the coupling ratio was measured as being close to unity, which means that the maximum physical velocity, $V_{\max}$, can be obtained by multiplying the maximum kinetic rate, $K_{\max}$, by the size of the step (8 nm). However, at high loads, the randomness cannot be considered constant any more, because of the measured values. Furthermore, r is also interpreted to be inversely proportional to the mean velocity (that decreases with F). In our case, the randomness is

proportional to $[I]_F$, so it has to diverge from $[I]_F$. What we think is that this kinetic model can deal with the coupling ratio in another way. Once we know that the inhibition is competitive, we can say that the ATP hydrolysis is the less inhibited step. Then it seems reasonable that, at high loads, the consumption of ATP can keep taking place without much difficulty, while no effective motion is observed. Maybe this is a hint that the mechano-chemical coupling is no more valid near the maximum force, but more refined data and a detailed description of the motor's mechanism are needed to confirm this fact.

DISCUSSION

The hypothesis that an external force acts as a mixed competitive inhibitor leads to theoretical predictions in good agreement with experimental data. This provides a kinetic inhibitory scenario that allows us to make accurate velocity predictions in the presence of a mechanical force, either assisting or opposing. The randomness at high [ATP] can also be well predicted. The mixed character of the inhibition leads to the conclusion that different chemical states have different sensitivities to the force. The competitive character is probably a clue to think that the mechano-chemical coupling is not conserved at high loads.

Furthermore, the analysis of the present paper constitutes a useful tool to study other experimental results obtained for different molecular motors. We believe that this work balances quite well the quality of the predictions with a minimum set of meaningful parameters, providing interesting clues to understand better the still-to-be-uncovered mechanisms of microtubule walking.

Finally, in our opinion, the most original result of this approach is not the good agreement with experimental data, but the methodology to establish the force dependence of the different chemical states. This can stimulate more precise theoretical models for kinesins and other molecular motors.

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REFERENCES

1 Schnitzer, M. J. and Block, S. M. (1997) Kinesin hydrolyses one ATP per 8-nm step. Nature (London) **388**, 386–390
2 Visscher, K., Schnitzer, M. J. and Block, S. M. (1999) Single kinesin molecules studied with a molecular force clamp. Nature (London) **400**, 184–189
3 Dixon, M. and Webb, E. C. (1979) Enzyme inhibition and activation. In Enzymes, 3rd edn. (Dixon, M., Webb, E. C., Thorne, C. J. R. and Tipton, K. F., eds.), pp. 332–467, Longman, London
4 Palmer, T. (1995) Understanding enzymes, 4th edn., Prentice Hall/Ellis Horwood, London
5 Schnitzer, M. J., Visscher, K. V. and Block, S. M. (2000) Force production by single kinesin motors. Nat. Cell Biol. **2**, 718–723
6 Fisher, M. E. and Kolomeisky, A. B. (2001) Simple mechanochemistry describes the dynamics of kinesin molecules. Proc. Natl. Acad. Sci. U.S.A. **98**, 7748–7753
7 Jülicher, F., Ajdari, A. and Prost, J. (1997) Modelling molecular motors. Rev. Mod. Phys. **69**, 1269–1281
8 Howard, J. (1997) Molecular motors: structural adaptations to cellular functions. Nature (London) **389**, 561–567
9 Svoboda, K and Block, S. M. (1994) Force and velocity measured for single kinesin molecules. Cell **77**, 773–784
10 Coppin, C. M., Pierce, D. W., Hsu, L. and Vale, R. D. (1997) The load dependence of kinesin's mechanical cycle. Proc. Natl. Acad. Sci. U.S.A. **94**, 8539–8544
11 Howard, J., Hudspeth, A. J. and Vale, R. D. (1989) Movement of microtubules by single kinesin molecules. Nature (London) **342**, 154–158
12 Bustamante, C., Chemla, Y. R., Forde, N. R. and Izhaky, D. (2004) Mechanical processes in biochemistry. Annu. Rev. Biochem. **73**, 705–748
13 Cortés, A., Cascante, M., Cárdenas, M. L. and Cornish-Bowden, A. (2001) Relationships between inhibition constants, inhibitor concentrations for 50 % inhibition and types of inhibition: new ways of analysing data. Biochem. J. **357**, 263–268
14 Block, S. M., Asbury, C. L., Shaevitz, J. W. and Lang, M. J. (2003) Probing the kinesin reaction cycle with a 2D optical force clamp. Proc. Natl. Acad. Sci. U.S.A. **100**, 2351–2356