

# A mathematical model describing the mechanical kinetics of kinesin stepping

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## ABSTRACT

**Motivation:** Kinesin is a smart motor protein that steps processively forward and backward along microtubules (MTs). The mechanical kinetics of kinesin affecting its stepping behavior is not fully understood. Here, we propose a mathematical model to study the mechanical kinetics of forward and backward stepping of kinesin motor based on the four-state discrete stochastic model of the motor.

**Results:** Results show that the probabilities of forward and backward stepping can be modeled using the mean probabilities of forward and backward kinetic transitions, respectively. We show that the backward stepping of kinesin motor starts when the probability of adenosine diphosphate (ADP) binding to the motor is much higher than that of adenosine triphosphate (ATP) binding. Furthermore, our results indicate that the backward stepping is related to both ATP hydrolysis and synthesis with rate limiting factor being ATP synthesis. Low rate of ATP synthesis under high backward loads above 10 pN is also suggested as a reason for the detachment of kinesin motor from MT in the kinetic state MT-Kinesin-ADP-P<sub>i</sub>.

**Availability and implementation:** The code for this work is written in Visual C# and is available by request from the authors.

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## 1 INTRODUCTION

Kinesin-1, which will be referred to as kinesin hereafter, is a two-headed motor protein that advances unidirectionally along a microtubule (MT) in discrete 8.2 nm steps (Clancy *et al.*, 2011) by walking in an asymmetric hand-over-hand fashion (Yildiz *et al.*, 2004, 2008). Kinesin can processively step forward and backward along an MT (Carter and Cross, 2005), taking approximately 100 steps before detaching from the MT (Clancy *et al.*, 2011). Experimental investigation of kinesin processivity has revealed that kinesin can undergo discrete steps in the presence of fuel, i.e. adenosine triphosphate (ATP), or in the absence of ATP, if external load is applied to the motor (Yildiz *et al.*, 2008).

The significance of studying the mechanical kinetics underlying stepping of kinesin motor becomes apparent when considering the important roles the motor plays ranging from human diseases to synthetic nanotechnological applications and the development of novel active nanodevices. Molecular biology have revealed critical roles of kinesin motor in brain wiring and formation, neuronal survival, higher brain function and control of central

nervous system (Hirokawa *et al.*, 2010). It is also evident that the impairment of these functions of kinesin motor would contribute to neuronal disease pathogenesis, such as Alzheimer (Stokin *et al.*, 2005) and hereditary spastic paraplegia (Ebbing *et al.*, 2008), and other diseases such as cancer (Yu and Feng, 2010). Kinesin stepping can also exert localized forces on nanostructures. This force can be exploited in nanorobotics (Hess, 2006). Thus, engineers have commenced to invent synthetic nanodevices powered by kinesin motor that have unique potentials for use in medical diagnostics as well as engineering applications (Fischer *et al.*, 2009; Goodman *et al.*, 2012; Hess, 2011; Kumar *et al.*, 2013; Sanchez *et al.*, 2012). However, precise temporal and spatial control of such nanodevices is a challenging problem because of the stochastic motion, high complexity and small size of kinesin (Khataee and Ibrahim, 2012; Khataee *et al.*, 2013; Wang and Manesh, 2010). Even though the biophysical properties of kinesin motor have been widely studied, yet little is known about how the mechanical kinetics of kinesin affect its stepping behavior in the complex cellular environment (Verhey *et al.*, 2011). Better understanding of the mechanical kinetics of kinesin stepping may translate into the development of molecular-targeted therapy for various human diseases (Hirokawa *et al.*, 2010; Yu and Feng, 2010) and precise motion control for artificial nanomotors with a wide variety of missions (Wang and Manesh, 2010).

Mathematical modeling can be used to not only conceptualize and characterize kinesin behavior under different experimental settings, but also to make predictions for novel applications. One major theoretical approach for the modeling of kinesin motor motion is based on a multistate discrete stochastic model and the assumption that a single stepping of the motor involves passing through a sequence of discrete kinetic states connected by reversible transition rates (Fisher and Kolomeisky, 2001; Kolomeisky and Fisher, 2007). Here, we mathematically model and analyze the mechanical kinetics of ATP-driven stepping behavior of kinesin motor over a discrete stochastic model at various forward (negative) and backward (positive) loads and ATP concentrations ([ATP]) at room temperature (forward and backward steps are taken toward the plus and minus ends of MT, respectively). We show that our mathematical model can be used to analyze, conceptualize and predict forward and backward stepping of kinesin motor in different situations.

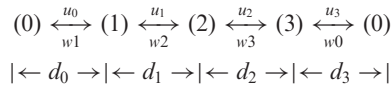
## 2 RESULTS

### 2.1 Mathematical model

For kinesin's processive movement, the simplest discrete stochastic model reported by Fisher and Kolomeisky (2001) defines a

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single stepping cycle of the motor as passing through a sequence of four kinetic states where state ( $i$ ) may transit to states ( $i + 1$ ) and ( $i - 1$ ) with forward and backward transition rates  $u_i$  and  $w_i$ ,  $i = 0, 1, 2, 3$ , respectively:



In this discrete stochastic model, the intermediate kinetic states of the model are denoted as M·K, M·K·ATP, M·K·ADP·P<sub>i</sub> and M·K·ADP, respectively, where M·K designates MT-kinesin complex and ADP·P<sub>i</sub> complex stands for the products of ATP hydrolysis: adenosine diphosphate (ADP) and inorganic phosphate (P<sub>i</sub>). The  $d_i$  represents the substep length for the center of force of kinesin along MT, where the total step size is  $d = (d_0 + d_1 + d_2 + d_3)$ .

It has been shown that the rates of transitions between kinetic states are influenced by the temperature  $T$  (Leibler and Huse, 1993) and external load  $F$  (Fisher and Kolomeisky, 2001) as:

$$u_i^{(F)} = u_i^{(0)} \exp\left(-\frac{\theta_i^+ Fd}{k_B T}\right) \quad (1)$$

$$w_i^{(F)} = w_i^{(0)} \exp\left(+\frac{\theta_i^- Fd}{k_B T}\right) \quad (2)$$

where  $u_i^{(0)}$  and  $w_i^{(0)}$  are the transition rates at zero load,  $\theta_i^+$  and  $\theta_i^-$  are the load distribution factors,  $d$  is the step size (equal to 8.2 nm) and  $k_B$  is the Boltzmann constant. The load distribution factors demonstrate how the external load affects the individual kinetic rates where it is assumed that  $\sum_{i=0}^3 (\theta_i^+ + \theta_i^-) = 1$ . These factors are related to substep lengths via  $d_i = (\theta_i^+ + \theta_{i+1}^-)d$  (Kolomeisky and Fisher, 2007). The parameter values of Equations (1) and (2) summarized in Table 1 as well as the parameter values of [ATP]-dependent rates presented in Equations (3) and (4) have been estimated by Fisher and Kolomeisky (2001) through regression on the experimental data of Block *et al.* in Schnitzer *et al.* (2000) and Visscher *et al.* (1999) at room temperature:

$$u_0^{(0)} = k_0[\text{ATP}] \quad (3)$$

$$w_0^{(0)} = \frac{k'_0[\text{ATP}]}{\sqrt{1 + \frac{[\text{ATP}]}{c_0}}} \quad (4)$$

where  $k_0 = 1.8 \mu\text{M}^{-1}\text{s}^{-1}$ ,  $k'_0 = 0.225 \mu\text{M}^{-1}\text{s}^{-1}$  and  $c_0 = 16 \mu\text{M}$ .

The four-state discrete stochastic model asserts that forward stepping of kinesin motor is associated with ATP hydrolysis, whereas backward stepping could give rise to ATP synthesis. Our mathematical model indicates that the backward stepping of kinesin motor is related to both ATP hydrolysis and synthesis. In discussing backward stepping under high backward loads, Carter and Cross (2005) stated that backward stepping is unlikely to result in ATP synthesis. However, Fisher *et al.* (2005) remarked that the experimental observations in Carter and Cross (2005) rest on a misconception of the significance of dwell times before forward and backward stepping, and asserted that backward stepping could result in ATP synthesis. In addition,

**Table 1.** Estimated parameters of forward and backward transition rate equations (Fisher and Kolomeisky, 2001)

State $i$	$u_i^{(0)} (\text{s}^{-1})$	$w_i^{(0)} (\text{s}^{-1})$	$\theta_i^+$	$\theta_i^-$
0	—	—	0.120	0.430
1	580	40	0.020	0.130
2	290	1.6	0.020	0.130
3	290	40	0.020	0.130

Yildiz *et al.* (2008) have shown that kinesin motor can step in the direction of the applied external load in the absence of ATP due to the strain on the kinesin head generated by the external load. Although the external load was able to trigger stepping in the absence of ATP, our work focuses on the ATP-driven stepping of kinesin motor and we study the effect of [ATP] and loads on the forward and backward stepping. [ATP] is considered as a factor that affects the motor velocity at various loads (Schnitzer *et al.*, 2000; Visscher *et al.*, 1999) and thus, it affects the mean waiting time over kinetic states modeled in this work. In several recent works, [ATP] is proposed as a mean for temporal control of synthetic nanodevices powered by kinesin motor (Dinu *et al.*, 2007; Wang and Manesh, 2010).

Equations (1) and (2) show that the rates of forward and backward transitions change under different loads, [ATP] and temperatures. We expect this rate change to change the probabilities of triggering these transitions, as well as the mean waiting time and the probability distribution over each kinetic states. We define the probabilities of forward and backward transitions in terms of the forward and backward transition rates as follows:

$$P(u_i) = \frac{u_i^{(F)}}{u_i^{(F)} + w_i^{(F)}} \quad (5)$$

$$P(w_i) = \frac{w_i^{(F)}}{u_i^{(F)} + w_i^{(F)}} \quad (6)$$

where  $i = 0, 1, 2, 3$ . Using the forward and backward transition rates, the mean waiting time in kinetic state  $s_i$  ( $i = 0, 1, 2, 3$ ) can be defined as follows:

$$\langle t(s_i) \rangle = \frac{1}{u_i^{(F)} + w_i^{(F)}} \quad (7)$$

Equation (7) defines the mean waiting time over the four kinetic states of kinesin motor during forward and backward stepping processes. We also define the stationary distribution of probability over each kinetic states of kinesin motor using the Hill's diagram method (Kurka and Dvorak, 1982), where a kinetic state change diagram is represented as a graph. Let  $G$  be a connected and directed graph with vertex set  $V(G)$  and edge set  $E(G)$ , which represent kinetic states and transitions of the four-state discrete stochastic model of kinesin motor, respectively. The edge weights of  $G$  correspond to transition rates of the discrete

stochastic model. The stationary distribution of probability over a vertex  $v \in V(G)$  is defined as follows:

$$P(v) = \frac{\sum_v}{\sum_{total}} \quad (8)$$

where  $\sum_v = \sum \{\prod_D | D \text{ is a directional diagram of } v\}$  is the sum of products of all directional diagrams of vertex  $v$ ;  $\prod_D = \prod \{e(m, n) | \{m, n\} \in V(D)\}$  is the product of edge weights of a directional diagram  $D$ ;  $e(m, n)$  is the edge weight between vertices  $m$  and  $n$ ; and  $\sum_{total}$  is the sum of all  $\sum_v$ .  $D$  is defined as a directional diagram of a vertex  $v$  if for any vertex  $x \in V(D)$  and  $x \neq v$ , there is exactly one path from  $x$  to  $v$ . Collectively, the mathematical model of the mechanical kinetics of stepping behavior of kinesin motor protein shows the behavior of the motor changes in response to changes in the environment.

## 2.2 Analysis and discussion

Experimental determinations reported the stall force of kinesin motor as a load ranging between 5–7.5 pN (Carter and Cross, 2005; Nishiyama *et al.*, 2002; Visscher *et al.*, 1999; Yildiz *et al.*, 2008). It was observed in Carter and Cross (2005) and Nishiyama *et al.* (2002) that (i) below stall, forward stepping of kinesin motor is dominant, (ii) at stall, the forward and backward stepping are equally likely and (iii) above stall, backward stepping is more frequent. To model the probabilities of forward and backward stepping of kinesin motor, we define the mean probabilities of forward and backward kinetic transitions as follows:

$$\langle P(u) \rangle = \sum_{i=0}^3 \frac{P(u_i)}{4} \quad (9)$$

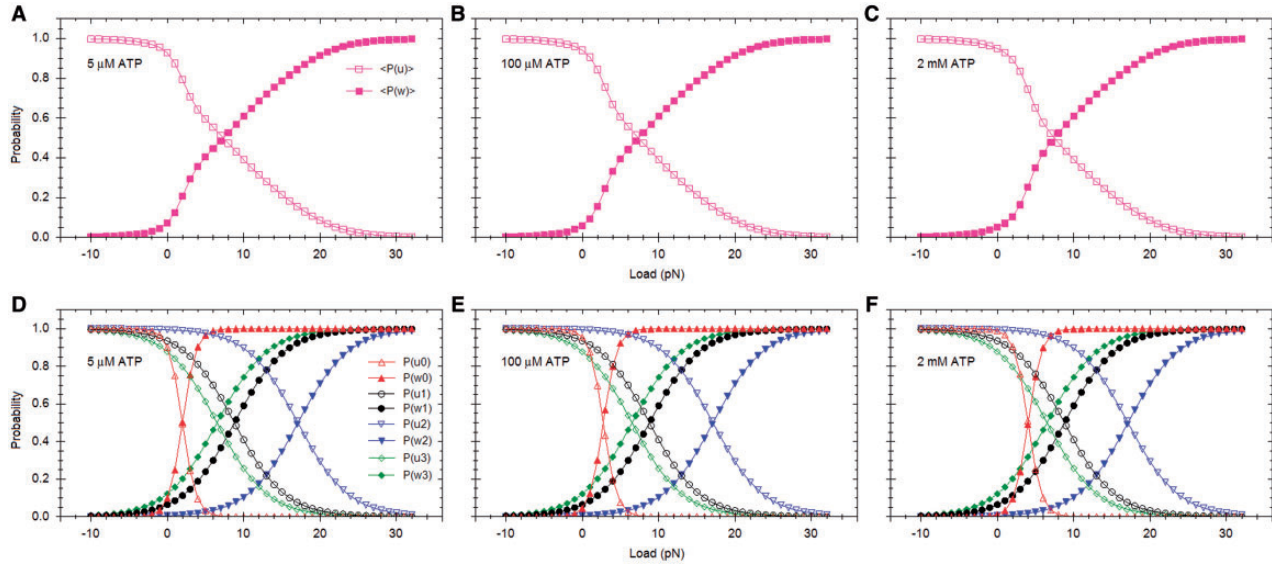
$$\langle P(w) \rangle = \sum_{i=0}^3 \frac{P(w_i)}{4} \quad (10)$$

where  $P(u_i)$  and  $P(w_i)$  are the probabilities of forward and backward transitions given by Equations (5) and (6). Figure 1A–C shows the mean probabilities of forward and backward kinetic transitions at loads over the range  $-10 \text{ pN} \leq F \leq 32 \text{ pN}$ . We define load  $F = -10 \text{ pN}$  as the supremum forward load where at this point and beyond,  $\langle P(u) \rangle > 0.99$  and  $\langle P(w) \rangle < 0.01$ . Similarly, the supremum backward load is defined at load  $F = 32 \text{ pN}$  where at this point and beyond,  $\langle P(w) \rangle > 0.99$  and  $\langle P(u) \rangle < 0.01$ . From Figure 1A–C, we observe that with increasing load from forward to backward, the mean forward transition probability  $\langle P(u) \rangle$  decreases from almost 1 and intersects with the mean backward transition probability  $\langle P(w) \rangle$  at load between 7–8 pN, beyond which  $\langle P(w) \rangle$  becomes dominant. Therefore, the stall force ( $F_{stall}$ ) of kinesin motor can be obtained by solving equation  $\langle P(u) \rangle = \langle P(w) \rangle$  at various [ATP]. Our results show an almost constant and [ATP]-independent stall force of  $F_{stall} \sim 7.3 \text{ pN}$ , in agreement with the experimentally measured stall force between 5–7.5 pN (Carter and Cross, 2005; Nishiyama *et al.*, 2002; Visscher *et al.*, 1999; Yildiz *et al.*, 2008). These results are also consistent with the experimental observations that stall force of kinesin motor is independent of [ATP] (Carter and Cross, 2005; Clancy *et al.*, 2011). Our obtained  $F_{stall}$  also agrees with the experimental results of Nishiyama *et al.* (2002) in which the probabilities of forward and backward stepping intersect at load

around 7–8 pN. Figure 1A–C also indicates that the mean probabilities of forward and backward transitions are almost [ATP]-independent. This is consistent with the observed [ATP]-independent but load dependent probabilities of forward and backward stepping by Nishiyama *et al.* (2002). It was reported that under forward loads of  $-15 \text{ pN} \leq F \leq -2 \text{ pN}$  only forward stepping was observed (Carter and Cross, 2005), but with increasing backward loads the probability of observing backward stepping increases (Nishiyama *et al.*, 2002). These experimental observations agreed with our results in Figure 1A–C as over the forward load ranging from  $-10 \text{ pN} \leq F \leq -2 \text{ pN}$ ,  $\langle P(u) \rangle > 0.98$  and  $\langle P(w) \rangle < 0.02$ , whereas with increasing backward load the probability  $\langle P(w) \rangle$  increases. In addition, our calculations show that at loads over the range  $0 \leq F \leq 8 \text{ pN}$  and at various [ATP],  $\langle P(u) \rangle$  decreases from  $> 0.95$  to  $\sim 0.48$  (see Fig. 1A–C). This is in good agreement with the reported probabilities for forward stepping of 1 and 0.5 at the respective loads of  $F = 0 \text{ pN}$  and  $F = 8 \text{ pN}$  by Taniguchi *et al.* (2005). These agreements of our model with experimental findings indicate that the probabilities of forward and backward stepping of kinesin motor can be modeled well using the mean probabilities of forward and backward transitions defined in Equations (9) and (10), respectively.

To further analyze the probabilities of forward and backward stepping of kinesin motor using kinetic transitions, we separately examine the probabilities of forward and backward kinetic transitions given by Equations (5) and (6) (see Fig. 1D–F). Figure 1D–F shows that with increasing load from high forward to backward loads at all [ATP],  $P(w_0)$  is the first backward probability function that intersects its corresponding forward probability function  $P(u_0)$ . As only transition rates  $u_0^{(F)}$  and  $w_0^{(F)}$  are [ATP]-dependent, the minimum load at which  $P(u_0)$  intersects with  $P(w_0)$  at [ATP] = 5  $\mu\text{M}$  is  $F = 1.987 \text{ pN}$  (Fig. 1D). At [ATP] = 100  $\mu\text{M}$  and [ATP] = 2 mM, the crossover load points of  $P(u_0)$  and  $P(w_0)$  are found to be at  $F = 2.754 \text{ pN}$  and  $F = 4.034 \text{ pN}$ , respectively (Fig. 1E and F). These results indicate that at limiting [ATP] the probability of forward stepping is almost equal to 1 when the applied load is  $< 1.987 \text{ pN}$  because at loads  $F < 1.987 \text{ pN}$  all the probabilities  $P(u_i)$  are greater than  $P(w_i)$ ,  $i = 0, 1, 2, 3$  (see Fig. 1D). Similarly, at high [ATP] almost all steps are made in the forward direction when the applied load is  $< 4.034 \text{ pN}$  (see Fig. 1F). This is in good agreement with the experimental observations of Nishiyama *et al.* (2002) that at loads lower than 4 pN the probability of forward stepping is almost equal to 1. In analyzing the probabilities of [ATP]-independent kinetic transitions, we explore their respective forward and backward kinetic transitions. Equation (1) is capable of showing that with increasing load from high forward to backward,  $u_2^{(F)}$  and  $u_3^{(F)}$  rates fall equally, as they have the same parameters (see Table 1), whereas they are lower than the decreasing  $u_1^{(F)}$ , indicating that the rate limiting step of the ATPase cycle is  $P_i$  or ADP release in the presence of MTs (Higuchi *et al.*, 1997). However,  $w_2^{(F)}$  rises with a rate much smaller than  $w_3^{(F)}$  because  $w_2^{(0)} = 1.6 \text{ s}^{-1}$  is small compared with  $w_3^{(0)} = 40 \text{ s}^{-1}$ , meaning that  $P_i$  binding rate is faster than the ATP synthesis rate, as reported by Hackney (2005). Thus, according to Equation (5), due to low rate of ATP synthesis, i.e.  $w_2^{(F)}$ , the probability of  $P_i$  release





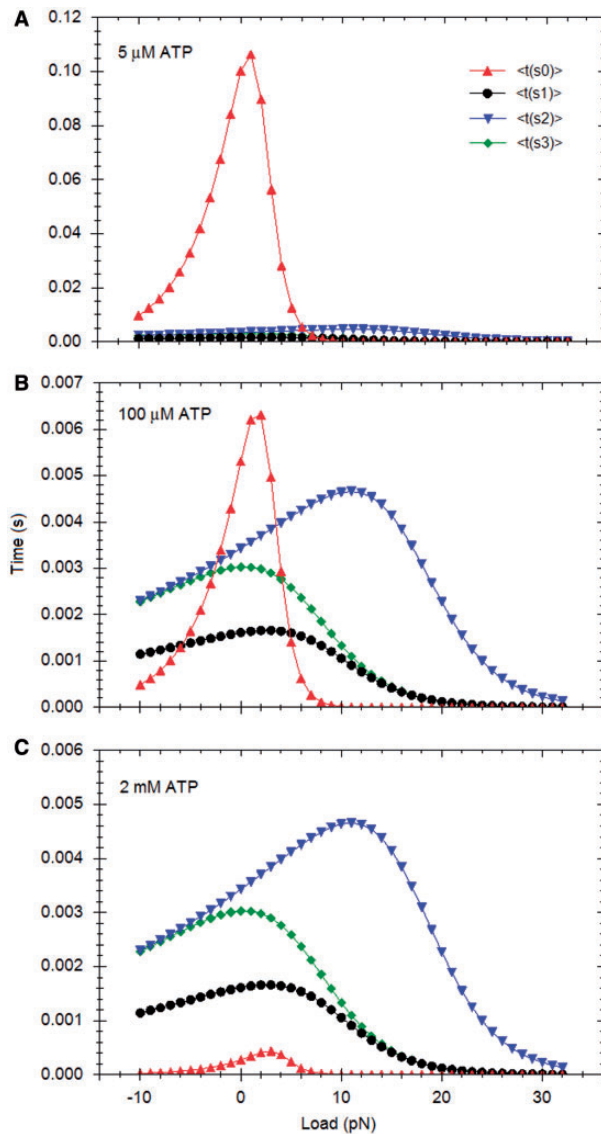
**Fig. 1.** Probabilities of kinetic transitions versus load at different [ATP]. (A–C) Mean probabilities of forward and backward kinetic transitions,  $\langle P(u) \rangle$  and  $\langle P(w) \rangle$ . (D–F) Probabilities of the forward and backward transitions,  $P(u_i)$  and  $P(w_i)$  where  $i=0, 1, 2, 3$

dominates the probability of ADP release, i.e.  $P(u_2) \geq P(u_3)$ , with a large difference. The low probability of ADP release agrees with the results of Hackney in Hackney (1988, 2005) where they observed that the rate limiting factor of ATPase activity of kinesin motor is the ADP release. Also, as  $w_3^{(F)}$  is equal to  $w_1^{(F)}$ ,  $P(u_1)$  falls between  $P(u_2)$  and  $P(u_3)$ , indicating that  $P_i$  release is at least as fast as ATP hydrolysis (Hackney, 2005). Similarly, the slow rise of  $w_2^{(F)}$  leads to lowering the rise of  $P(w_2)$  in comparison with  $P(w_3)$ , confirming that  $P_i$  binding rate is faster than the ATP synthesis rate (Hackney, 2005).  $P(w_1) \geq P(w_2)$  also indicates that the rate of ATP dissociation is higher than that of ATP synthesis, as  $w_1^{(F)} \geq w_2^{(F)}$ .

The forward and backward stepping processes of kinesin motor start from kinetic state '0' by the forward and backward transitions rates  $u_0^{(F)}$  and  $w_0^{(F)}$ , respectively. As forward stepping of kinesin motor switch to backward at loads  $F > F_{stall}$ , we examine  $P(u_0)$  and  $P(w_0)$  at  $F = F_{stall}$ . Our calculations show that  $P(w_0) > 0.97$  and  $P(u_0) < 0.03$  at load range  $F > F_{stall}$ . This result implies that the backward stepping of kinesin motor starts when the probability of ADP binding to the motor is much higher than that of ATP binding. The crossover load points of probabilities of [ATP]-independent kinetic transitions are also calculated at loads: (i)  $F = 6.515$  pN for  $P(u_3)$  and  $P(w_3)$ , (ii)  $F = 8.795$  pN for  $P(u_1)$  and  $P(w_1)$  and (iii)  $F = 17.102$  pN for  $P(u_2)$  and  $P(w_2)$ . However, we are not able to relate these crossover load points with the available experimental data because in current experiments, [ADP] and [P<sub>i</sub>] are not separately measured (Fisher and Kolomeisky, 2001). The result obtained by separately examining the probabilities of [ATP]-independent kinetic transitions illustrates that the probability of ATP hydrolysis,  $P(u_1)$ , is higher than that of ATP synthesis,  $P(w_2)$ , at loads  $F_{stall} < F < 13$  pN. At this load range, Figure 1A–C indicates that the backward stepping of the kinesin motor is more probable than forward stepping. This result suggests that the backward stepping of kinesin

motor is related to ATP hydrolysis as experimentally observed in Carter and Cross (2005) and Nishiyama *et al.* (2002).

What gives rise to the [ATP]-dependent detachment of kinesin from MT? Equation (7) enables us to model the mean waiting time over the four kinetic states of kinesin motor during forward and backward stepping processes at different [ATP] and loads (Fig. 2). The results show that at [ATP] = 5 μM and low loads the mean waiting time in kinetic state '0' is greater than that of other states and peaks at a maximum of 0.1055 s at  $F = 1.148$  pN in comparison with a maximum of 0.0063 s at  $F = 2$  pN and [ATP] = 100 μM (see Fig. 2A and B). These results are consistent with experimental findings as at limiting [ATP], kinesin is paused at M-K kinetic state, waiting for ATP binding (Clancy *et al.*, 2011; Hackney, 1994). It has been observed by Schnitzer *et al.* (2000) that at limiting [ATP] the probability of detachment of kinesin motor from MT in kinetic state '0' increases with the increase in the duration of this state, whereas at high [ATP] the probability of detachment from kinetic state '0' diminishes. Our results in Figure 2A and B indicate that the load points that maximize  $\langle t(s_0) \rangle$  are close to the crossover load points of  $P(u_0)$  and  $P(w_0)$  at [ATP] = 5 μM and 100 μM. Thus, these results indicate that at low [ATP] the probability of detachment from kinetic state '0' is the highest when the probabilities of ATP and ADP binding to the motor are close. Figure 2C also shows that at high [ATP], the ATP binding rate rises, which leads to a reduction in the duration of kinetic state '0' and an increase in the durations of kinetic states '2' and '3'. This agrees with the findings of Schnitzer *et al.* (2000) that at high [ATP] the probability of detachment from kinetic state '0' diminishes. Accordingly, our results suggest states '2' and '3' as the most probable kinetic states for the detachment of kinesin motor from MT at high [ATP] and low loads, in agreement with the reported kinetic states for the detachment of kinesin from MT (Hackney, 2002; Schliwa, 2003; Seitz and Surrey, 2006; Yajima *et al.*, 2002). The detachment of kinesin motor from MT under high backward



**Fig. 2.** The mean waiting time over the four kinetic states,  $\langle t(s_i) \rangle$  where  $i=0, 1, 2, 3$ , versus load at different [ATP]

loads has been observed more commonly at loads  $>10$  pN,  $F_{detach} > 10$  pN (Carter and Cross, 2005). Therefore, our results in Figure 2A–C show that at high backward loads  $>10$  pN and all [ATP] the mean waiting time in kinetic states ‘2’ and ‘3’ is greater than that of other kinetic states. These results suggest states ‘2’ and ‘3’ as the first and second most probable kinetic states for the detachment of kinesin motor from MT at loads  $>10$  pN, in agreement with the reported kinetic states for the detachment of kinesin from MT (Schliwa, 2003; Seitz and Surrey, 2006).

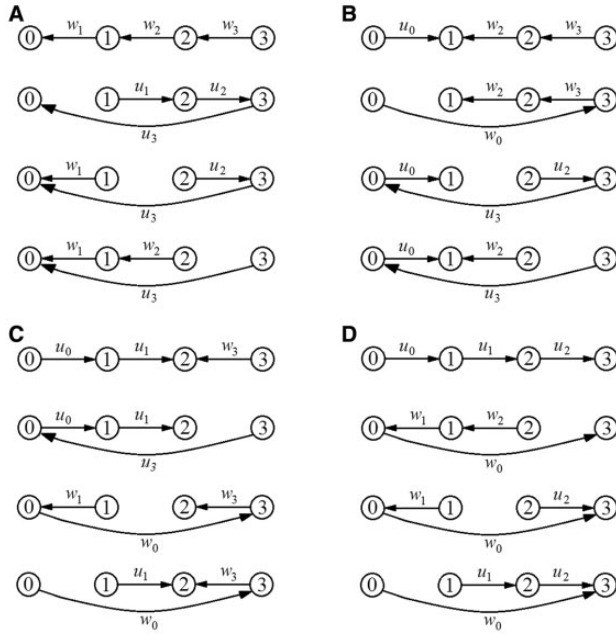
To explain the higher durations of kinetic states ‘2’ and ‘3’, we analyze their respective forward and backward kinetic transitions. As we discussed in analyzing the probabilities of [ATP]-independent kinetic transitions, with increasing load from high forward to backward: (i) forward  $u_2^{(F)}$  and  $u_3^{(F)}$  rates fall

**Table 2.** Ratio of  $\langle t(s_2) \rangle$  to  $\langle t(s_i) \rangle$  at  $F=10.946$  pN, where  $i=0, 1, 3$

ATP (μM)	$\langle t(s_2) \rangle / \langle t(s_i) \rangle$		
	$i=0$	$i=1$	$i=3$
5	63.654	5.067	4.200
100	541.704	5.067	4.200
2000	2599.708	5.067	4.200

equally and (ii) backward  $w_2^{(F)}$  rises with a rate much smaller than  $w_3^{(F)}$  [see Equations (1) and (2)]. Thus, according to Equation (7), the slow rise of ATP synthesis rate, i.e.  $w_2^{(F)}$ , is the reason that  $\langle t(s_2) \rangle$  attains its maximum at higher loads and at a time that is higher than that of  $\langle t(s_3) \rangle$ . Figure 2A–C indicates  $F=10.946$  pN as the load point that the mean waiting time in kinetic state ‘2’ is the highest,  $\langle t(s_2) \rangle = 0.0046$  s for all [ATP], due to the slow rise of  $w_2^{(F)}$ . This implies that at high backward loads  $F > F_{stall}$  the rate limiting factor of kinesin’s backward stepping is ATP synthesis,  $w_2^{(F)}$ .

To compare the duration of kinetic state ‘2’ with that of other kinetic states at load  $F=10.946$  pN, we compute the ratio of  $\langle t(s_2) \rangle = 0.0046$  s to  $\langle t(s_i) \rangle$  using Equation (7), where  $i=0, 1, 3$  (see Table 2). Table 2 shows that the duration of kinetic state ‘2’ is at least 63 times greater than that of kinetic state ‘0’ at load  $F=10.946$  pN and [ATP]=5 μM. With increasing [ATP], the duration of kinetic state ‘0’ decreases, which leads to a rise in the ratio of  $\langle t(s_2) \rangle$  to  $\langle t(s_0) \rangle$ . As the forward and backward kinetic transitions of states ‘1’ and ‘3’ are [ATP]-independent, the ratio of the duration of kinetic state ‘2’ to those of kinetic states ‘1’ and ‘3’ at load  $F=10.946$  pN are constant, at 5.067 and 4.200, respectively (see Table 2). Hence, in accordance with the observations of Schnitzer *et al.* (2000), our results in Table 2 suggest  $F=10.946$  pN as the load point where the probability of detachment of kinesin motor from MT in kinetic state ‘2’ is the highest. This is consistent with the common detachment load  $F_{detach} > 10$  pN observed by Carter and Cross (2005). As we showed that at loads  $F_{stall} < F \leq F_{detach}$  the long mean waiting time in kinetic state ‘2’ is due to the low transition rate  $w_2^{(F)}$ , this low rate of ATP synthesis can be proposed as a reason for the detachment of kinesin motor from MT in kinetic state ‘2’. Furthermore, Figure 2A–C shows that at loads  $F > 10.946$  pN the mean waiting time in state ‘2’ decreases because of the fast rise of ATP synthesis rate,  $w_2^{(F)}$ . Thus, based on these results, it can be deduced that with an increase in the rate of ATP synthesis at high backward loads  $F > F_{stall}$ , the long duration of being in kinetic state ‘2’ would decrease, which leads to the reduction of the detachment probability of kinesin motor from MT. The rise of ATP synthesis rate may lead to increase in the velocity of backward stepping of kinesin motor under high backward loads. These results and the results obtained by separately examining the probabilities of [ATP]-independent kinetic transitions (see Fig. 1D–F) conclude that the backward stepping of kinesin motor is related to both ATP synthesis and hydrolysis. The duration of state ‘2’ decreases to almost 0 at loads  $F \geq 32$  pN as in



**Fig. 3.** Directional diagrams of kinetic states ‘0’–‘3’ depicted in parts (A–D), respectively. For clarity, the transitions rates  $u_i^{(F)}$  and  $w_i^{(F)}$  are depicted as  $u_i$  and  $w_i$ , respectively, where  $i=0, 1, 2, 3$

this load range the rate of ATP synthesis,  $w_2^{(F)}$ , is at least 93 times greater than the rate of  $P_i$  release,  $u_2^{(F)}$ .

Obviously, the duration of kinetic states in the forward and backward stepping processes of kinesin motor should agree with the stationary distribution of probability in these states. According to Equation (8), to compute the stationary distribution of probability over kinetic states, it is required to obtain the directional diagrams of the states. We derive the directional diagrams of four kinetic states using the definition of directional diagram given in the previous Section (see Fig. 3).

For example, Figure 3A shows four directional diagrams of kinetic state ‘0’, which result in four products and their sum as follows:

$$\prod_0 = \{w_3 w_2 w_1, u_1 u_2 u_3, u_2 u_3 w_1, u_3 w_2 w_1\} \quad (11)$$

$$\sum_0 = (w_3 w_2 w_1 + u_1 u_2 u_3 + u_2 u_3 w_1 + u_3 w_2 w_1) \quad (12)$$

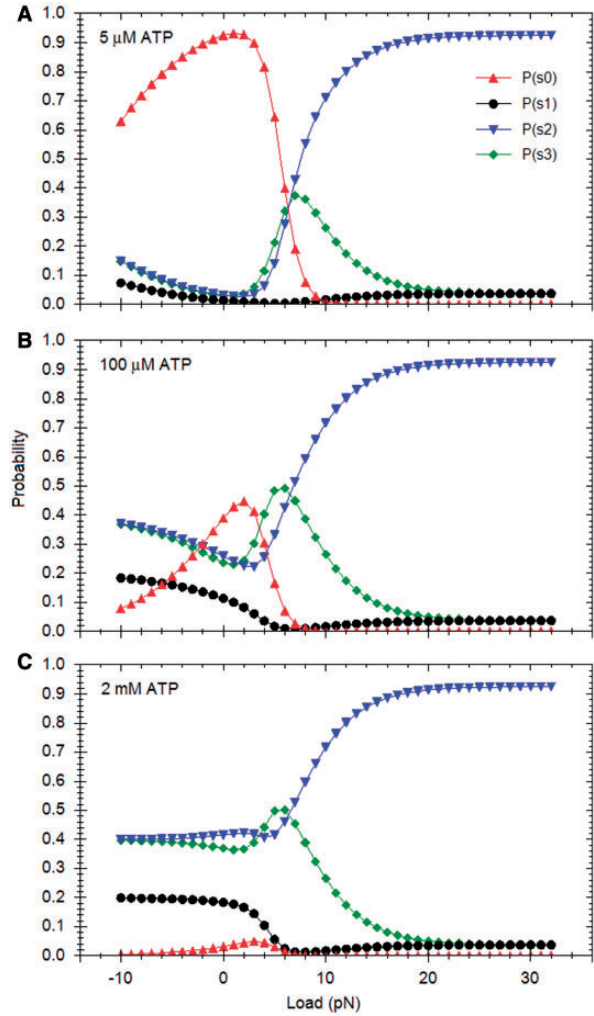
Similarly,  $\prod_i$  and  $\sum_i$ , where  $i=1, 2, 3$ , are obtained from Figure 3B–D, respectively.  $\sum_{total}$  is the sum of all 16 products obtained from four states:

$$\sum_{total} = \sum_0 + \sum_1 + \sum_2 + \sum_3 \quad (13)$$

Therefore, we define the distribution of probability over kinetic states  $s_i$  ( $i=0, 1, 2, 3$ ) as follows:

$$P(s_0) = \frac{w_3 w_2 w_1 + u_1 u_2 u_3 + u_2 u_3 w_1 + u_3 w_2 w_1}{\sum_{total}} \quad (14)$$

$$P(s_1) = \frac{u_0 w_3 w_2 + w_0 w_3 w_2 + u_0 u_2 u_3 + u_3 u_0 w_2}{\sum_{total}} \quad (15)$$



**Fig. 4.** Distribution of probability over the four kinetic states,  $P(s_i)$  where  $i=0, 1, 2, 3$ , versus load at different [ATP]

$$P(s_2) = \frac{u_0 u_1 w_3 + u_3 u_0 u_1 + w_1 w_0 w_3 + u_1 w_0 w_3}{\sum_{total}} \quad (16)$$

$$P(s_3) = \frac{u_0 u_1 u_2 + w_2 w_1 w_0 + u_2 w_1 w_0 + u_1 u_2 w_0}{\sum_{total}} \quad (17)$$

where  $u_i$  and  $w_i$  ( $i=0, 1, 2, 3$ ) are computed by Equations (1) and (2). Using Equations (14)–(17), the distribution of probability over the four kinetic states is plotted in Figure 4 and shows a similar pattern to the mean waiting time over these states in Figure 2. As it can be seen from Figure 4A, at low [ATP] and low loads the distribution of probability in kinetic state ‘0’ is the highest. This is consistent with the obtained results of the mean waiting time over kinetic state ‘0’ and confirms that at limiting [ATP] kinesin mainly stays in kinetic state ‘0’ and waits for ATP binding (Clancy *et al.*, 2011; Hackney, 1994). Figure 4A and B shows that the distribution of probability in kinetic state ‘0’ peaks at maximum points of 0.93 and 0.43 at loads  $F=1.987$  pN and  $F=2.754$  pN, respectively, coincide with the crossover



load points of  $P(u_0)$  and  $P(w_0)$  at  $[ATP] = 5 \mu\text{M}$  and  $100 \mu\text{M}$ . These results imply that the probability of observing kinesin motor in kinetic state '0' will be the highest when the probabilities of ATP and ADP binding to the motor are equal, i.e.  $P(u_0) = P(w_0)$ . This also confirms our earlier results about the duration over kinetic state '0'. At high  $[ATP]$  and low loads, the plot shows that the distribution of probability in kinetic states '2' and '3' becomes dominant (see Fig. 4C), in agreement with the obtained results of the durations of kinetic states '2' and '3' in Figure 2C. It is also seen from Figure 4A–C that at high backward loads  $>10$  pN and at all  $[ATP]$ , the distribution of probability in kinetic states '2' and '3' is greater than that of other kinetic states. This suggests '2' and '3' as the first and second most probable kinetic states that kinesin motor stays at loads  $>10$  pN, in agreement with the obtained results of the durations of these kinetic states and the reported kinetic states for the detachment of kinesin from MT (Schliwa, 2003; Seitz and Surrey, 2006). The high distribution of probability in kinetic state '2' at high loads  $F_{\text{stall}} < F \leq F_{\text{detach}}$  confirms the long duration of this state due to the low rate of ATP synthesis.

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