

# Cooperativity in the motor activities of the ATP-fueled molecular motors

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## Abstract

Kinesin, myosin and  $F_1$ -ATPase are multi-domain molecular motors with multiple catalytic subunits. The motor mechanochemicals are achieved via the conversion of ATP hydrolysis energy into forces and motions. We find that the catalysis of these molecular motors do not follow the simple Michaelis–Menten mechanism. The motor activities, such as the hydrolysis or processive rates, of kinesin, myosin and  $F_1$ -ATPase have a complex ATP-dependent cooperativity. To understand this complexity in kinetics and mechanochemicals, we develop a conformation correlation theory of cooperativity for the ATP-fueled motor proteins. The quantitative analysis and simulations indicate that cooperativity is induced by the conformational coupling of binding states of different subunits and prevails in the motor activities.

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## 1. Introduction

Kinesin, myosin and  $F_1$ -ATPase are regarded as molecular motors for generating cellular forces and motion by transducing catalytic chemical energy. In recent years, various motor proteins have been discovered and harnessed for the purpose of single molecule manipulation and nanobiotechnology [1]. Mechanically, kinesin (walking hand-over-hand along microtubules) [2,3] and myosin (dragging and rowing along actin filaments) are linear stepping motors [4,5], and ATP synthase ( $F_1F_0$ -ATPase, rotating while transducing hydrolysis/synthesis of ATP molecules) is a rotary spinning motor [6–8]. Despite the wide spectrum of behavior, all of these motors are very similar in the generation of mechanics. The basic mechanochemical mechanisms involve the hydrolysis of nucleotides (mainly ATP and its analogues), inducing the conformational changes and the structural transitions further into motion and other mechanics of the active subunits.

For various families of kinesins, myosins and  $F_1$ -ATPases, the motors are structurally multi-domain (multi-subunit) and catalytically multi-site ATPase proteins. They were found to be dimers (for most of kinesins and myosins) [3,4,9–12] or hexamers (for  $F_1$ -ATPases) [6,13–15]. Conventional kinesin (Fig. 1A) and myosin (Fig. 1B) are dimers that interlace the reaction cycles of two catalytic heads [3,4,9–12]. For a fraction of the ATPase cycle, one head is tightly bound to the track of a microtubule or an actin filament. The two heads can be either in-phase or out-of-phase and are in different conformation states. The functionally stable  $F_1$ -ATPase [6,13–15] (Fig. 1C) is composed of three alternative catalytic sites on ( $\alpha\beta$ ) hexamer subunits. Three catalytic sites are located on the  $\beta$  subunits at the interfaces with the  $\alpha$  subunits. Three ( $\alpha\beta$ ) subunits are in different binding states during the ATPase cycle. The molecular basis of motor operation of kinesin, myosin and  $F_1$ -ATPase are the sequential conformational changes of the active subunits at different scales triggered and powered by the ATP hydrolysis reactions.

In the steady operation of ‘bi-site’ (kinesin and myosin) and ‘tri-site’ ( $F_1$ -ATPase) molecular motors, there is a highly coordinated action between different subunits in

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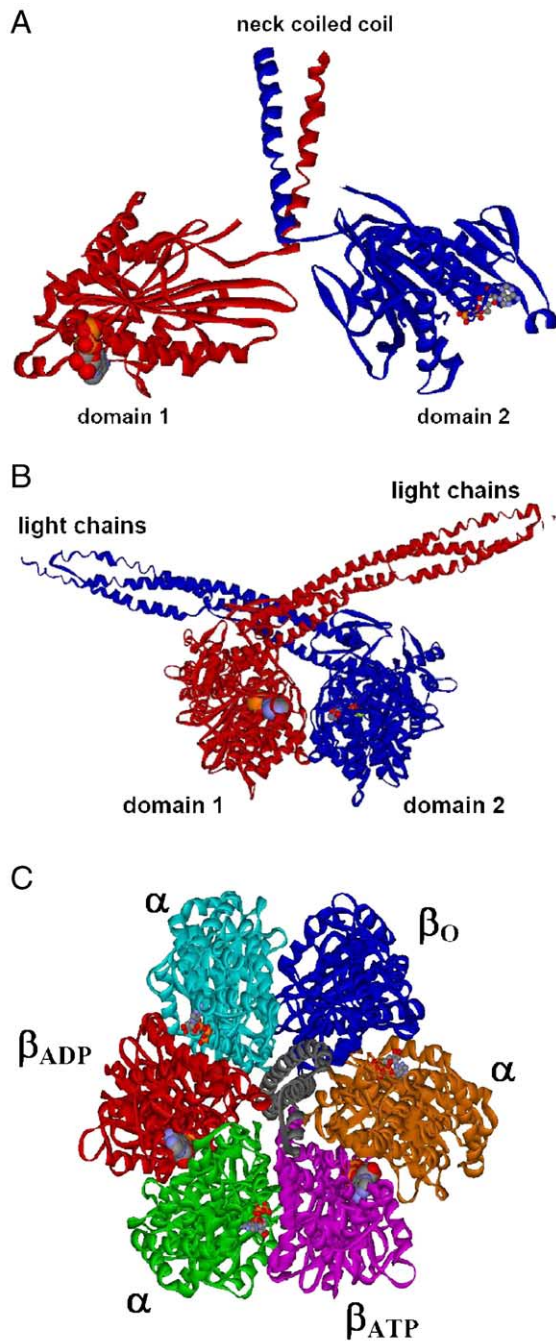


Fig. 1. Structural conformations of multi-site catalysis in (A) dimeric kinesin (Protein Data Bank code: 3KIN), (B) dimeric myosin (Protein Data Bank code: 1G8X) and (C) hexameric F<sub>1</sub>-ATPase (Protein Data Bank code: 1H8H).

catalysis activities and in conformational changes. For example in F<sub>1</sub>-ATPase, three ( $\alpha\beta$ ) catalysis sites are in

## 2. Conformational correlation theory of cooperativity

### 2.1. Cooperativity of motor activity

The cooperativity of ligand binding and enzyme catalysis is a common manifestation of allostery. The allosteric activity and function of proteins underlie oligomeric and asymmetric conformational changes (e.g., binding-state changes), which are

different conformers at any given time. The alternative conformations and catalytic states at three ( $\alpha\beta$ ) pairs work in sequential collaboration (Fig. 1C). This means that while one site tightly binds ATP and undergoes hydrolysis, the next one loosely binds the hydrolyzed ADP, and the third one opens to release the products and intake ATP. The collaborative and synchronized conformational changes in ( $\alpha\beta$ )<sub>3</sub> induce a torque between the hexamer ( $\alpha\beta$ )<sub>3</sub> and the central stalk  $\gamma$  subunit, causing the F<sub>1</sub>-ATPase motor to rotate [6,16]. Some questions [13] have been raised concerning the multi-site binding-change schemes and the cooperativity of hydrolysis reactions at multiple catalytic subunits. A fundamental issue which remains unanswered is whether or not a collaborative and synchronized mechanism is universally maintained. If there is a breakdown of this mechanism, disordered actions of the subunits would lead to complex behavior of enzyme kinetics and result in cooperativity of motor activities [19]. In fact, both negative and positive cooperativity have been reported [17–19].

Currently, a molecular understanding of the cooperative mechanism of ATP catalysis in kinesin, myosin and F<sub>1</sub>-ATPase is not available. Enormous efforts have been made to resolve the nature of the operating mechanism and mechanochemical couplings, such as single molecule experiments [3,7,8,20–22], mechanochemical simulation [23–25] and atomistic molecular dynamics studies [26–28]. However, the interactive cooperativity from different ATP catalytic subunits has neither been comprehensively determined nor fully understood at the molecular level. It has been shown that kinesin and myosin have structural mechanisms for cooperativity between the two head domains [46]. Previously, we proposed [19,25] a molecular model of the F<sub>1</sub>-ATPase motor based on enzyme kinetics and Langevin dynamics. Our quantitative analysis [19] indicated that in F<sub>1</sub>-ATPase, complex cooperativity of ATP hydrolysis occurred. The origins of such cooperativity remain unknown and needs to be explored. In this work, we show that complex cooperativity also occurs in the kinesin and myosin motors. To attempt to understand the cooperativity of motor activities in the multi-subunit kinesin, myosin and F<sub>1</sub>-ATPase, we develop a conformational correlation (CC) theory. The CC theory is demonstrated to work well and leads to further molecular insights into the coupling mechanism and operating dynamics of the ATP-fueled molecular motors.

normally triggered by the substrates/ligand molecules and dynamic interactions. In the multi-subunit motor proteins, the subunit–subunit interactions and binding of ligands can alter the dynamic courses of conformational changes. This can result in cooperativity between subunits, i.e., binding, occupancy or catalysis reaction at one site affects the binding of additional ligands to the neighboring subunits. Allosteric cooperativity occurs when the binding of one ligand increases (positive cooperativity) or decreases (negative cooperativity) the enzyme activity of a multi-subunit protein for binding subsequent ligands [29,30]. In terms of structural changes, the conformational state of each subunit influences its neighbors and there is a correlation of conformation between subunits. In the case of positive cooperativity, ligand binding to one site facilitates ligand binding to other sites of the enzyme. For negative cooperativity, ligand binding to one site inhibits ligand binding to other sites.

The physical basis of cooperativity is the inter-subunit interactions during catalysis and other actions. Cooperativity is by nature a dynamical process [31]. Several theories have been developed to explain cooperativity in enzyme proteins [30]. The widely accepted models are the concerted model of Monod–Wyman–Changeux (MWC) [32] and the sequential ligand-induced model of Koshland–Némethy–Filmer (KNF) [33]. The KNF model is more general and realistic because it does not assume a sharp coordinated switch from one state to the other but rather assumes successively ordered steps. In contrast to the MWC model, KNF can explain both negative and positive cooperativity. Nevertheless, both MWC and KNF models are limited to simple cases of cooperativity. Recently, a conformational spread (CS) model was proposed [34] to describe the general allosteric effects spreading through proteins of multi-states. This theory is difficult to apply to systems as complex as the heterotropic motor proteins. In view of these considerations, developing new concepts of cooperativity is of particular importance for molecular motors.

## 2.2. Quantitative expression of cooperativity

From a conventional kinetics point of view, we can quantitatively describe the enzymatic cooperativity in the ATP-fueled molecular motors by a modified Hill equation [35],

$$\log(R/(R_{\max} - R)) = h \log[ATP] - \log K \quad (1a)$$

where  $R_{\max}$  is the saturation hydrolysis rate (or saturated motor speed if applicable),  $K$  is a constant and  $[ATP]$  is the concentration of the ATP molecules. In Eq. (1a)  $h$  is the Hill number and is determined from the slope of the Hill plot of  $\log(R/(R_{\max} - R))$  versus  $\log[ATP]$ .

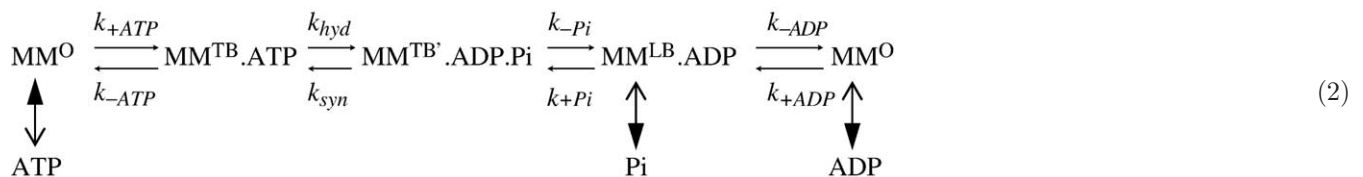
From Eq. (1a), we derive a more accurate numerical determination of  $h$ ,

$$h = \frac{R_{\max}[ATP]}{R(R_{\max} - R)} \times \frac{dR}{d[ATP]} \quad (1b)$$

When  $h = 1$ , there is no cooperative interaction between different catalytic sites; only one ATP molecule is hydrolyzed at any time and the kinetics of motor proteins follows the Michaelis–Menten mechanism, i.e.  $R = R_{\max}[ATP]/(K_m + [ATP])$  with  $K_m$  the Michaelis constant. When  $h > 1$ , there is positive cooperative hydrolysis between different subunit sites and more than one ATP molecules are simultaneously undergoing catalysis reactions. Values of  $h < 1$  indicate negative cooperative behavior where there might be some inhibition between the multi catalysis sites.

## 2.3. Conformational correlation (CC) theory of cooperativity

In the ATP-fueled molecular motors, a conformational change to the structural elements caused by ATP hydrolysis at the ATP-binding site is communicated to either the next corresponding track-binding site (for kinesin and myosin) or to the alternative binding site (for  $F_1$ -ATPase). It is then relayed at much large spatial scales via homotropic structural elements to a mechanical amplifier of the active subunits [6,12]. The cytoplasmic pathways are the same: a complete enzymatic cycle of ATP hydrolysis in kinesin, myosin and  $F_1$ -ATPase occurs via the following pathway [1,3–5,11,19,21,36–38],



where MM refers to the enzymatic molecular motor. The binding conformational states of the multiple catalytic subunits are identified as tight binding (TB, bound with ATP), loose binding (LB, bound with ADP and/or Pi), and Open (O), respectively. The open (O) site has a very low affinity for substrates and is catalytically inactive.  $k_{+ATP}$ ,  $k_{-ATP}$  (or  $k_{+ADP}$ ,  $k_{-ADP}$  and  $k_{+Pi}$ ,

$k_{-pi}$ ) refer to the rate constants of association and dissociation of ATP (or ADP and Pi) molecules to the motor, i.e., they are the rate of ATP (or ADP and Pi) binding to or unbinding from active subunits. The terms  $k_{hyd}$ ,  $k_{syn}$  are the hydrolysis and synthesis rate constants of ATP. In this catalysis pathway, the direction of hydrolysis resulting in binding/unbinding to the motor of ATP, ADP and Pi is given by the solid arrow heads, whereas the thin arrow heads point to the synthesis direction. In each hydrolysis cycle, the different subunits interact in a cooperative way as the moving domains sequentially contact and interact with each other. The binding and conformational changes are highly coupled to the ATP hydrolysis cycle at different catalysis sites [25]. For example in F<sub>1</sub>-ATPase, the multi-site hydrolysis at three ( $\alpha\beta$ ) catalytic subunits are believed to be biochemically equal [6,16,39]. In steady cycles of ATP hydrolysis reactions, the hydrolysis reactions at three sites could be regarded as a three-fold fast equilibrium process with the same reaction sequence and dynamics.

As demonstrated in our previous work [19], the steady state ATP hydrolysis cycles of Eq. (2) can be interpreted by a series of fast equilibrium reactions. The equilibrium equations in this case were written for the binding and hydrolysis of ATP molecules in the motor proteins taken at a time. Generally in enzyme kinetics, we define the steady activity rate of an  $N$  binding-site motor as,

$$R = \frac{\sum_{i=0}^N iL_i[ATP]^i}{\sum_{i=0}^N L_i[ATP]^i} = \frac{\partial \log \Sigma}{\partial \log [ATP]} \quad (3)$$

where  $\Sigma = \sum_i L_i [ATP]^i$ ,  $L_0 = 1$  and  $L_i = k_1 k_2 \dots k_i$  is the product of the associate constants for the binding of ATP to the first site, the second site and so on. Virtually all activity of the multi-site motor proteins can be described by Eq. (3), but this is not a meaningful exercise unless the kinetics and function of individual binding sites are measurable.

In the absence of catalysis cooperativity, we can derive that the steady-state condition of Eq. (2) gives the overall rate [19] of ATP hydrolysis at given physiological conditions (such as, of given [ATP], [ADP] and [Pi]) by,

$$R = \frac{(-k_{-ATP}k_{syn}k_{ADP}[ADP]k_{Pi}[Pi] + k_{-ADP}k_{-Pi}k_{hyd}k_{ATP}[ATP]) / (k_{-ADP}k_{-Pi}k_{hyd} + (k_{hyd}k_{-Pi} + k_{-ATP}k_{-Pi} + k_{-ATP}k_{syn} + (k_{-ATP}k_{syn} + k_{ADP})k_{Pi}[Pi])k_{ADP}[ADP] + k_{hyd}(k_{-ADP} + k_{-Pi} + k_{Pi}[Pi])k_{ATP}[ATP] + (k_{-ADP}k_{-Pi} + k_{-ADP}k_{syn} + k_{syn}k_{Pi}[Pi]) \times (k_{-ATP} + k_{ATP}[ATP]))}{(4)}$$

In the limit of product concentrations, this is actually a Michaelis–Menten mechanism,  $R = R_{max}[ATP]/(K_m + [ATP])$ , which means there is no increased (positive cooperativity) or decreased (negative cooperativity) activities from different binding sites. However, the symmetric subunit structures and even identical catalysis kinetics do not guarantee that the catalysis at multiple sites takes place in a highly ordered manner. To check the effect of increased/decreased interactions between the catalytic sites of molecular motors, we shall carefully investigate the cooperativity in its kinetics or motor mechanochemicals.

Assuming there are  $N$  structurally symmetric and catalytically identical subunits in the motor protein, each follows the same enzymatic pathways transiting in between different conformations (i.e., binding states). When the transitional interactions between adjacent subunits are constant, there is little correlation of conformations or binding states. In contrast, significant biased interaction between neighboring subunits causes the state of one subunit to influence its neighbors and there is a correlation of conformation between subunits.

Under biological conditions of motor proteins, the conformational transition of subunits is regulated by the binding of ligand molecules. For kinesin, myosin and F<sub>1</sub>-ATPase, ‘TB<sub>ATP</sub>’, ‘LB<sub>ADP</sub>’ and ‘Open’ are identified as three major conformation states (note that the TB’<sub>ADP,Pi</sub> binding state in Eq. (2) is a fast equilibrium state of TB<sub>ATP</sub>) [3,9,10,16,38,39]. The occurrence of multiple states of neighboring ‘Open’, ‘TB<sub>ATP</sub>’ and ‘LB<sub>ADP</sub>’ is entropically favored but penalized by the interactions between each other. The interactions are sufficient to lead to a correlated conformational change of states between subunits if their interaction energy ( $\Delta G$ ) exceeds a critical value ( $\Delta G_c$ ). When  $\Delta G \sim \Delta G_c$ , there would be a fluctuating transition between the states of ‘TB<sub>ATP</sub>’, ‘LB<sub>ADP</sub>’ and ‘O’.

Here, we propose the coupling energy due to correlation interaction at subunit  $i$  is,

$$\Delta G_c^i = - \sum_{j,j \neq i}^N c_{ij} S_i S_j \quad (5)$$

where  $S_i$  and  $S_j$  represents the corresponding binding states at subunit  $i$  and its neighboring subunit  $j$ . The product  $S_i S_j$  takes on values of 1, 0,  $-1$ , referring to the situation of positive, null or negative cooperativity between the states  $S_i$  and  $S_j$ ,

<sup>1</sup> If Eq. (3) is regarded as the first order function of  $\sum L_i [ATP]^i$ , we could have  $\frac{dR}{d \log [ATP]} = \langle R^2 \rangle - \langle R \rangle^2$  (where  $\langle \rangle$  symbols the mean). This indicates the variance or fluctuation of  $R$ .

respectively. The term  $c_{ij}$  is the correlation coefficient between states of subunits  $i$  and  $j$ . Given that the conformational correlation between subunits  $i$  and  $j$  is an isothermal entropy-driven process, we define on average,

$$c_{ij} \sim k_B T \ln \frac{(P_i - \sigma)(P_j + \sigma)}{P_i P_j} \quad (6)$$

Here  $\sigma$  is defined as the conformational correlation factor. It indicates how much one of the binding states  $S_i$  or  $S_j$  affects the other via the conformational correlated interactions. Physically it is the deviation of occupation probability difference ( $P_i - P_j$ ), with  $P_i$  or  $P_j$  the steady state occupation probability of binding-state  $S_i$  or  $S_j$ . Eq. (6) is justified by the conservation of binding-state distributions. To make this clear, we treat the probability difference,  $P_i - P_j$ , to have a standard normal distribution that satisfies the Gaussian distribution function,

$$\frac{1}{\sqrt{2\pi\sigma^2}} \int \exp \left[ -\frac{(x - (P_i - P_j))^2}{2\sigma^2} \right] dx = 1 \quad (7)$$

An extreme scenario is when  $\Delta G_c^i$  exceeds a critical interaction energy,  $E_{ij}$ , the neighboring subunit  $j$  would definitely be in (or not be in) the same state as subunit  $i$  due to conformation correlation,

$$\langle E_{ij} \rangle = k_B T \ln N \quad (8)$$

This is the all-or-none correlation energy with infinite subunits approximation [34]. The former case is equivalent to the WMC model and the latter one is the KNF situation.

When the correlated coupling between subunits affects the path and amplitude of their conformational changes, we have to consider two situations: in-pathway or out-of-pathway. Therefore, we propose that the conformational correlations eventually affect the motor activities through two kinds of schemes,

**Scheme I.** If the conformation correlation does work during the rate-limiting steps of pathways (i.e., of Eq. (2)), the kinetics rate constant is increased or decreased by the appropriate Arrhenius factor,

$$k_c = k_i^0 \exp \left[ -\frac{\Delta G_c^i}{k_B T} \right] \quad (9)$$

here  $k_c$  is the rate constant with cooperativity.  $k_i^0$  is the rate constant with no cooperativity considerations (as defined in Eq. (2)).

**Scheme II.** On the other hand, if the correlation induces a detour from the pathway (i.e. of Eq. (2)) and transits the motor into off-pathway states [20], the overall rate of activities would be changed by a generalized Boltzmann equation,

$$R_c = R^0 \prod_i \frac{1 + a_i}{1 + a_i \exp \left[ \frac{\Delta G_c^i}{k_B T} \right]}$$

$R^0$  is the overall rate constant without cooperativity (given as  $R$  in Eq. (4) in this case). The term  $a$  is a constant that indicates the degree to which the cooperativity affects the overall rate, i.e. for  $a \ll 1$ , there would be no cooperativity effect, but when  $a \geq 1$ , the cooperativity-induced coupling transition between the neighbor subunits are inevitable.

To apply the conformation correlation theory on the ATP-fueled molecular motors, we establish the following criteria,

- (1) multiple subunits are catalytically equal.  $S_i$  or  $S_j$  switches between the three major conformation states of ‘TB’, ‘LB’ and ‘Open’, as a function of physiological condition, e.g. [ATP];
- (2) conformation correlation may result in positive and negative coupling energy between states that increases (in particular for a pair of neighboring subunits having the same conformation) or decreases the rate of activities. As a result, a positive, null or negative cooperativity occurs;
- (3) no conformational correlation between the ‘Open’ state and other binding states. This is biochemically straightforward for enzyme proteins. The conformational correlation only induces interaction between different states of ‘TB’ and ‘LB’, and/or between ‘TB’, ‘LB’ themselves. The former interaction would result in negative coupling while the latter produce positive coupling;
- (4) the probability of occupation in each conformational state actually slightly change under different physiological (i.e. [ATP], [ADP], . . .) concentrations [19]. Thus, the term  $\sigma$  is a [ATP]-dependent constant in low [ATP] region but should be invariant in the steady operation region.

### 3. Experimental data and simulation procedures

#### 3.1. Conformation states of kinesin, myosin and F<sub>1</sub>-ATPase

Kinesin and myosin consist of two identical catalytic subunits linked by a small intermediate (hinge) subunit (Fig. 1). The hinge is an equatorial domain that binds substrates of ATP or ADP and is involved in most intra- and all inter-subunit contacts [9,12]. In F<sub>1</sub>-ATPase, the crystal structures of three ( $\alpha\beta$ ) pairs are almost identical and have a strong symmetry. The incorporation of the central subunits and substrates creates a structural and functional asymmetry between three catalytic sites [6,13,16]. In kinesin, myosin and F<sub>1</sub>-ATPase, the subunits have homologous structures and the enzymatic capabilities at each site are regarded equal. The active subunits exist in equilibrium between different conformational states, as we discussed in Section 2.3, and the binding states of each subunit have transitions between the tight binding (TB or TB'), loose binding (LB) and Open (O) states. The structural difference, e.g., among different kinesins, is neglected and this is justified by the kinetic rates of activities.

The cooperativity in molecular motors implies that a conformational change of the active subunit upon ATP binding/hydrolysis is coordinated into the energy-favored state by dynamic states of the remaining subunits [18,19,40,41]. Of course, there might be other mechanisms to trigger disorder states, such as ADP inhibition and ionic strength effects. These factors are negligible for the steady operating motors in a wide range of physiological conditions (e.g., of [ATP]). Generally speaking, cooperativity should extend to include the case of motor ensembles, e.g., in cell, myosin works as an ordered array of motors to move an actin filament. Conformation correlation can occur between the neighboring motor heads. Therefore, the CC theory also applies to the situation of an array of myosin II S1 constructs. We still investigate the possible difference of cooperativity between dimeric inter-head and monomeric intra-head.

#### 3.2. Steady-state activities and the CC analysis of cooperativity

The experimental data of steady motor activity of kinesin, myosin and F<sub>1</sub>-ATPase was collected and digitally reproduced from dimeric *Drosophila* K401-wt kinesin [11,36], dimeric *Dictyostelium* myosin-II [5,42] and hexameric *Bacillus* PS3 F<sub>1</sub>-ATPase [8,43], respectively. They represent the most comprehensive data available in terms of both the enzyme kinetics and motor activities. The activities data, i.e., the hydrolysis rates or the stepping/rotating rates, were originally presented in the form of a standard treatment (i.e., the rate was given versus [ATP]) and were analyzed by fitting to the Michaelis–Menten equation. To avoid errors in deducing the experimental data, the rates of kinetics/activities were reproduced in the format of reduced rate (or motor processive speed). We firstly analyzed the possible existence of kinetic cooperativity in these motors by constructing the Eadie–Hofstee plots [35]. Then, the Hill plots were generated to show the quantitative variation of cooperativity. The cooperativity described by the Hill number,  $h$ , was determined by the first-order deviation of experimental data via Eq. (1a). Alternatively for the simulations, the activity rate is derived by Eq. (4) and the cooperativity value of  $h$  was determined according to Eq. (1b).

From Eq. (2) through to Eq. (10), activity rate  $R$  and Hill number  $h$  are the motor quantities being analyzed against the kinetic inputs of [ATP], [ADP] and [Pi]. Rate constants  $k_i$  are values from experimental measurements. Detail values are listed in Table 1. Probability of occupation without cooperativity,  $P_i$  or  $P_j$ , is computed as performed in our previous work [19]. Only the conformation correlation factor  $\sigma$  and possible schemes of correlated coupling are simulation parameters. Every possible scheme and possibly affected conformer state due to the conformation correlation effect were cross-checked by fitting  $R$  vs. [ATP] to the experimental values. The value of the correlation factor,  $\sigma$ , and the scheme were finally determined from the optimized

Table 1  
The kinetics parameters of the ATP-fueled molecular motors

Molecular motor	Kinsin <sup>a</sup>		Myosin <sup>b</sup>		F <sub>1</sub> -ATPase <sup>c</sup>	
	<i>(Drosophila</i> K401wt)		<i>(Dictyostelium</i> myosin-II)		<i>(Bacillus</i> PS3)	
Rate constants of substrate association ( $M^{-1} s^{-1}$ ) and dissociation ( $s^{-1}$ ):	$k_{ATP}=1.1 \times 10^6$	$k_{-ATP}=200$	$k_{ATP}=6.1 \times 10^5$	$k_{-ATP}=64^d$	$k_{ATP}=2.08 \times 10^{7c}$	$k_{-ATP}=270$
	$k_{ADP}=n.d.$	$k_{-ADP}=200$	$k_{ADP}=2.4 \times 10^{5d}$	$k_{-ADP}=15^d$	$k_{ADP}=8.90 \times 10^6$	$k_{-ADP}=490$
	$k_{Pi}=n.d.$	$k_{-Pi}=40^f$	$k_{Pi}=n.d.$	$k_{-Pi}=250^f$	$k_{Pi}=8.10 \times 10^5$	$k_{-Pi}=2.03 \times 10^3$
Rate constants of binding changes of ATP hydrolysis and synthesis ( $s^{-1}$ ):	$k_{hyd}=100$	$k_{syn}=n.d.$	$k_{hyd}=160$	$k_{syn}=n.d.$	$k_{hyd}=4.5 \times 10^5$	$k_{syn}=1.15 \times 10^{-3}$
Conformation Correlation Factor, $\sigma$	0.07 ± 0.02		0.005 ± 0.003		0.12 ± 0.04	

<sup>a</sup> From ref. [11,36].

<sup>b</sup> From ref. [5,42].

<sup>c</sup> From ref. [51].

<sup>d</sup> From ref. [52].

<sup>e</sup> From ref. [8,43].

<sup>f</sup> Determined by simulation according to Eq. (4).

simulation that best fits to the experimental data. This is discussed in greater detail in Section 4.2.

### 3.3. Computing details

The computation and simulations of cooperativity, which involves the implementations of Eqs. (1a) and (1b) through to Eq. (10) in Section 2.3, were programmed using the Mathematica library [53]. The codes were compiled and executed on a standard pentium-4 PC or HP-Alpha workstation. The overall computing time is in the order of CPU minutes.

## 4. Results and discussion

### 4.1. Complex cooperativity in kinesin, myosin and $F_1$ -ATPase

Fig. 2 shows the Eadie–Hofstee plots of the reduced motor velocity/rate,  $V_R = V/V_{\max}$ , versus  $V_R/[ATP]$ , where  $V$  is the stepping/rotating speed or the rate of hydrolysis reactions and  $V_{\max}$  is the saturation value. As seen in Fig. 2, complex cooperativity of kinetics in the motor activities is apparent from the non-linear behavior of the Eadie–Hofstee plots of kinesin (triangles, Fig. 2A), myosin (circles, Fig. 2B) and  $F_1$ -ATPase (diamonds, Fig. 2C). Although the original literature analysis [5,8,11,36,42,43] explained the motor kinetics by fitting to the Michaelis–Menten equation, it could not be guaranteed that the catalysis follows the Michaelis–Menten mechanism (which is only justified by uni-site catalysis kinetics). A linear Eadie–Hofstee plot is indeed held for the Michaelis–Menten mechanism. Any subtle difference in the Michaelis–Menten fitting would result in large nonlinear changes in the corresponding Eadie–Hofstee plot. In the original analysis [5,8,11,36,42,43], the data points scattered away from the Michaelis–Menten fitting were not addressed thoroughly. Here we reproduce their original Michaelis–Menten fittings approximately by the dash lines in Fig. 2A, 2B and 2C). In the Eadie–Hofstee plots, these dash lines (Michaelis–Menten mechanism) are shown to poorly fit the experimental data. Therefore, the scattered data is not the experimental error as believed previously, but is more likely an indication of other mechanisms, such as cooperativity between subunits, involved in the catalysis reactions. Notably, the Michaelis–Menten fitting cannot give any information on possible cooperativity for the multi-site motors. On the other hand, the complex concave/convex Eadie–Hofstee plots, as of Fig. 2, show clearly the existence of cooperativity in motor activities.

The complex cooperativity appears to be a common characteristic for the ATP-fueled molecular motors. As seen in Fig. 3, the Hill plots of ATP hydrolysis rate (or motor speed),  $\log(R/(R_{\max} - R))$  versus  $\log[ATP]$ , show both positive and negative cooperativity of motor activities in

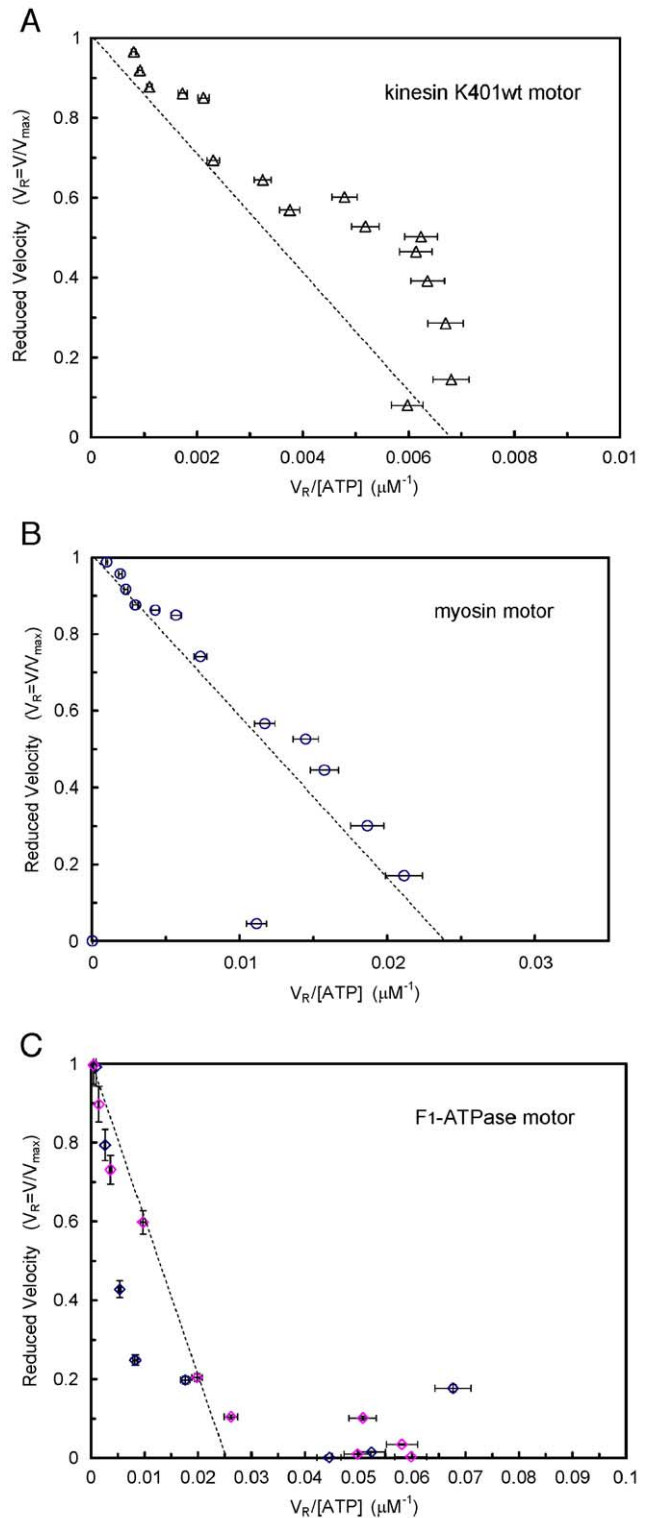


Fig. 2. Nonlinear Eadie–Hofstee distributions of experimental data in (A) kinesin ( $\Delta$ ) [11,36] (B) myosin (O) [5,42] and (C)  $F_1$ -ATPase ( $\diamond$ ) [8,43] indicating the existence of cooperativity of motor activity (hydrolysis rate or motor speed) in these ATP-fueled molecular motors. The dashed line is the simulation by a Michaelis–Menten mechanism.

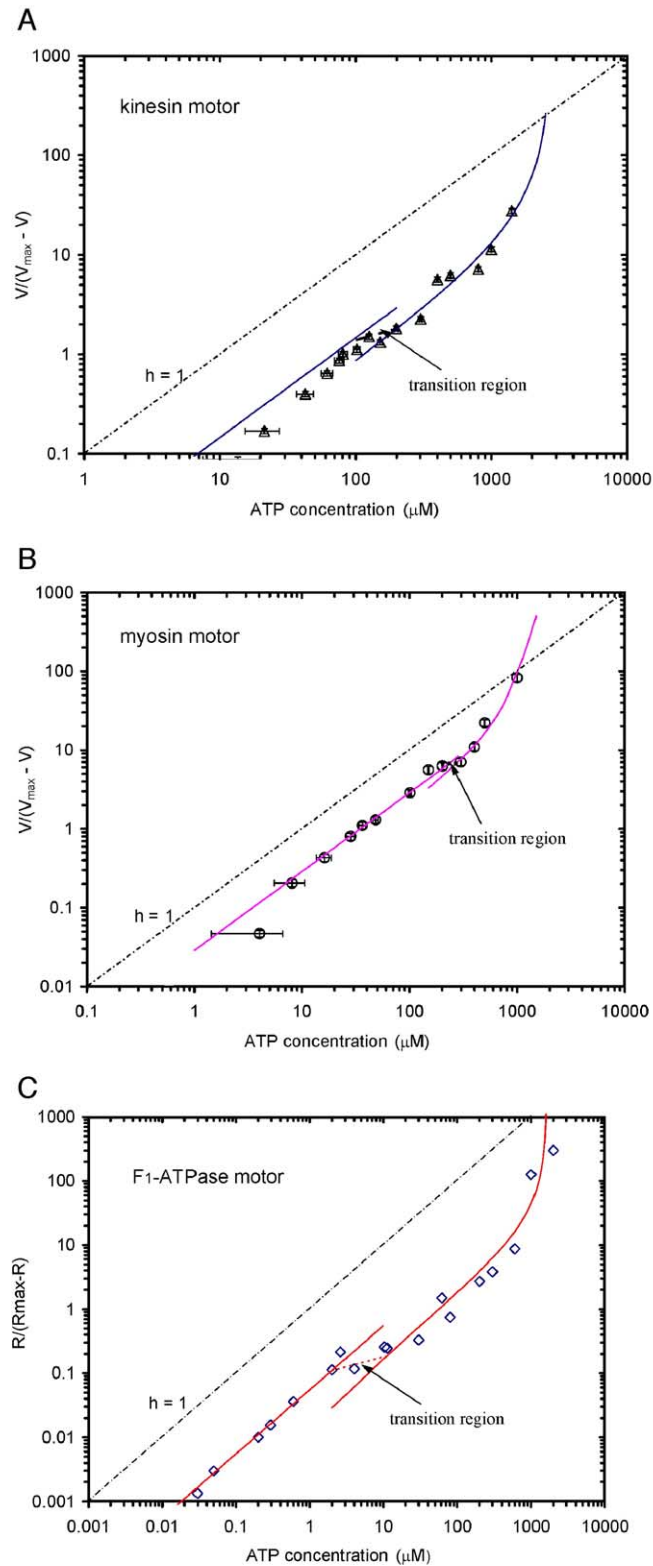


Fig. 3. Hill plots of ATP hydrolysis rate,  $\log(V/(V_{\max} - V))$ , versus  $\log[\text{ATP}]$  showing complex cooperativity of motor activities in (A) kinesin (B) myosin, and (C) F<sub>1</sub>-ATPase. The symbols refer to the experimental data. The solid lines are simulation results according to the conformational correlation theory, which indicates positive and negative cooperativity of ATP hydrolysis occurring across different [ATP] regions. Kinetic parameters of rate constants are given in Table 1 ([ADP] and [Pi] are set at cellular physiological condition of 10  $\mu\text{M}$  and 1.0 mM, respectively).

kinesin (Fig. 3A, triangles), myosin (Fig. 3B, circles) and F<sub>1</sub>-ATPase (Fig. 3C, diamonds). As with Fig. 2 (A, B and C), these experiments indicate that ATP hydrolysis or stepping processivity in molecular motors does not simply follow the Michaelis–Menten mechanism, which should be parallel to the dashed line and have  $h=1$ . There is a different cooperativity from multiple catalytic subunits occurring across the whole [ATP] region. From the Hill plots of the experimental data, the cooperativity of hydrolysis reactions in these ATP-fueled molecular motors can be distinguished by three concentration regions. Region 1, for the lower region (i.e. [ATP]=nM ~ 10  $\mu$ M for F<sub>1</sub>-ATPase, but up to 100  $\mu$ M for kinesin and myosin), the motor obeys Michaelis–Menten kinetics and there is no cooperativity in hydrolysis reactions. Region 2 is in the intermediate pre-saturated region (e.g. [ATP]=10  $\mu$ M ~ 100  $\mu$ M for F<sub>1</sub>-ATPase), and there is a negative cooperativity. In the saturated Region 3 (e.g. [ATP]>100  $\mu$ M for F<sub>1</sub>-ATPase), a positive cooperativity occurs for the hydrolysis reactions from different sites. In Region 1, a Michaelis–Menten mechanism is sufficient for understanding the motors' operation. However, from the pre-saturated Region 2 to the saturated Region 3, complex cooperativity of motor activities plays a dominant role over the simple Michaelis–Menten mechanism. Comparatively, Region 2 is much wider for F<sub>1</sub>-ATPase than for kinesin and myosin. In addition, a transition, shown as the dotted lines of Fig. 3A, 3B and 3C, comes from a null cooperativity (with  $h=1$ ) to the full cooperativity.

Fig. 4 shows the Hill number, as an indication of cooperativity, plotted against [ATP] in kinesin (triangles, Fig. 4A), myosin (circles, Fig. 4B) and F<sub>1</sub>-ATPase (diamonds, Fig. 4C). In Fig. 4, the experimental data are derived by Eq. (1a) and the simulation data is from the implementation of Eqs. (1b), (4) and (9). The dramatic variation of  $h$  values away from  $h=1$  demonstrates how the cooperativity changes its null, negative or positive properties within different concentration regions of ATP. It should be noted that there is error in deducing the Hill numbers (especially for those between  $h=0$  and 1) according to Eq. (1a) as the experimental data is very scattered. However, this error is not sufficiently large to invalidate the existence of positive, null or negative cooperativity crossing the whole ATP regions.

#### 4.2. Conformation correlation induced cooperativity of motor activities

To understand the complex cooperativity of motor activities, we introduced the concept of conformation correlation (CC) for the ATP-fuelled molecular motors (the theoretical concept is described in Section 2.3).

We use the fact that different catalytic subunits have the same pathway of hydrolysis reactions as Eq. (2). To date, there has been no report distinguishing the pathway difference among the multi-subunits of naturally occurring motor

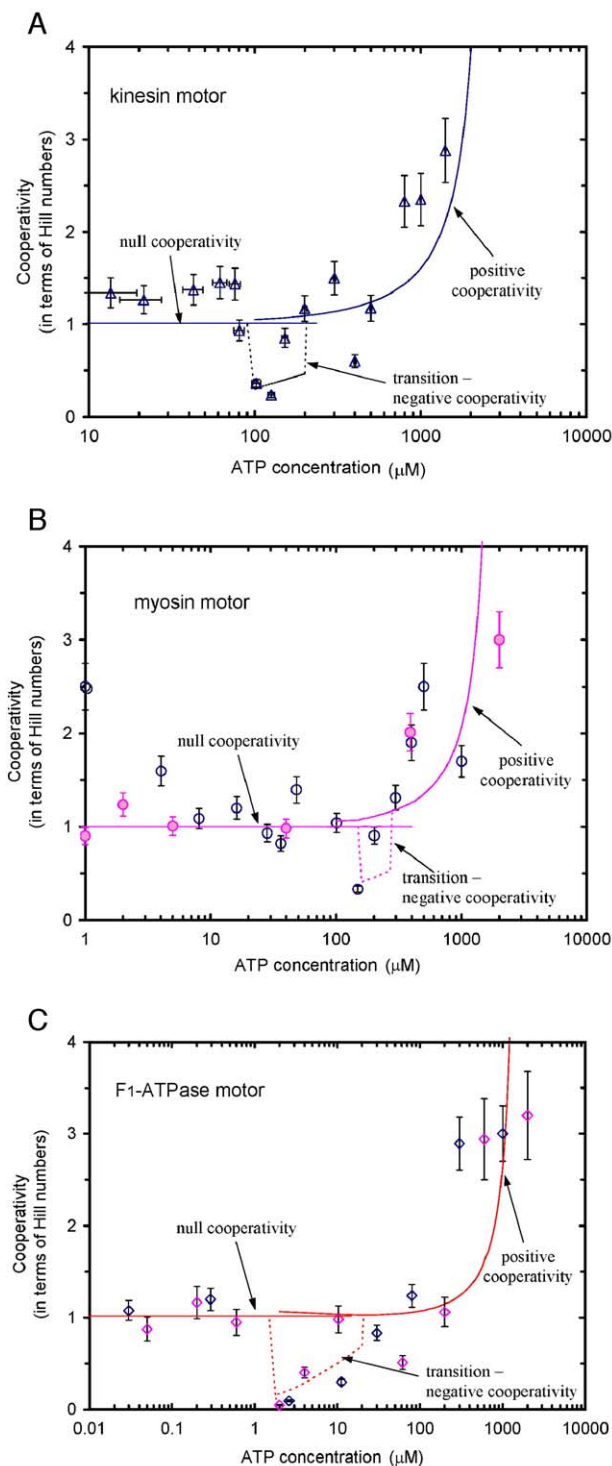


Fig. 4. Cooperativity changes versus [ATP]. The symbols refer to the derived value from experimental measurements of (A) kinesin ( $\Delta$ ), (B) myosin (O), and (C) F<sub>1</sub>-ATPase ( $\diamond$ ), respectively. The good fits of simulation (solid lines) from the conformational correlation theory indicate that the cooperativity is a fundamental mechanism of molecular motors.

proteins. The catalytic subunits are also assumed to have the same rate of association and dissociation of nucleotides of ATP, ADP.Pi or ADP. The simulations were carried out with the kinetics parameters listed in Table 1. [ADP] and [Pi] are

set at cellular physiological conditions of 10  $\mu\text{M}$  and 1.0 mM, respectively, for myosin-II and  $F_1$ -ATPase, but set at experimental conditions of 5  $\mu\text{M}$  of [ADP] and 1  $\mu\text{M}$  of [Pi] for kinesin K401wt.

Following the region classification from Section 4.1, the overall activity rates in Fig. 3 were simulated by two separate scenarios: no cooperativity for Region 1 and possible cooperativity for Regions 2 and 3. In addition, to account for the transition, shown as the dotted lines of Fig. 3A, 3B and 3C, from null-cooperativity to fully cooperative catalysis, we simply assume that the transition of enzyme kinetics takes place via a linear sampling path, with the sampling threshold linearly changing from 100% non-cooperativity to 100% cooperativity. The dotted lines, forming a kind of hysteresis loop, in the Hill number dependence (Fig. 4A, 4B and 4C) are the direct results from this transition. In Fig. 4, the amplitude and size of the hysteresis loops are a reflection of the sampling of experimental data in Fig. 3, i.e., the transitional [ATP] range and linear steps (in this work, we chose 100 steps), rather than anything else meaningful. Nonetheless, this does not affect the qualitative conclusion that the transition does occur.

When ATPs are hydrolyzed with possible cooperativity between different catalysis sites, the solid lines predicted by CC theory in Fig. 3 and Fig. 4 give a much better fit to the experimental data. This indicates that cooperativity of ATP catalysis induced from conformation correlation is the case for the kinesin, myosin and  $F_1$ -ATPase motors, at least for the saturated [ATP] region. From the simulations, it was found that kinesin, myosin and  $F_1$ -ATPase have a rate-limiting cooperativity, as regulated by Eq. (9). No motor has the off-pathway (Eq. (10)) cooperativity. Given the off-pathway transition is demonstrated to be a characteristic of the load-dependence velocity of the kinesin motor [20], this implies a fundamental difference for the external load dependence and the cooperativity from the internal conformation correlations.

We tested all possible rate-limiting effects with the CC schemes and find that,

- (a) for kinesin, strong correlation between M.ATP (plus M.ADP.Pi) and M.ADP affect all rate constants (Eq. (2)) except for the dissociation rate of ATP and Pi. Conformer M.ADP.Pi has the largest occupation probability of about 60% at saturated steady state. The conformation correlation factor is best-fitted as  $\sigma=0.07\pm 0.02$ . This means about 7% of TB or DB states are changed over due to conformation correlation;
- (b) for myosin-II, correlated interaction between M.ADP and M.ATP affects all forward reaction rates in its pathway and the association rate of ADP with a conformation correlation factor of  $\sigma=0.005\pm 0.003$ . M.ADP has the largest state occupation probability of about 80%;

- (c) for  $F_1$ -ATPase, M.ADP and M.ATP conformation correlation only affect the ATP association rate and the ADP dissociate rate. The M.ADP state is the highly occupied state with probability of about 60%. The conformation correlation factor is best-fitted as  $\sigma=0.12\pm 0.04$ .

Provided that kinesin, myosin and  $F_1$ -ATPase are structurally, functionally and mechanically different, one would expect to see some differences of cooperativity in these motors. In the CC theory, these differences affect the conformational changes and activities are interpreted by different interactive conformational correlation between the subunits. We find that the conformation correlations in kinesin and myosin are weaker than in  $F_1$ -ATPase, i.e., they have smaller conformational correlation factors. The molecular basis for different conformation correlations most likely lies in the different mechanochemical dwelling steps during these motors' ATPase cycle. At any given time, the time and strength of one subunit interacting with the neighboring ones would be different for different motors. For example, M.ADP and M.ADP.Pi are the strong binding-state for myosin and kinesin respectively, during which the motors' functional subunit attacks their tracks of actin filament or microtubule [3,5,12]. A major operating difference is that kinesin is attached to the track during its rate-limiting step of M.ADP.Pi [21] and myosin is detached from its tracks via M.ADP [10,38]. For  $F_1$ -ATPase, M.ADP is a tightly binding state. However, this is the most rate-limiting and dwelling state as the motor waits for Pi release upon a major conformational change of the active subunits [8,44,45].

In a similar manner in which the Michaelis–Menten equation underlies the uni-site kinetics and reaction equilibrium, CC theory shows that kinesin, myosin and  $F_1$ -ATPase have mechanisms in common for the cooperation of different subunits in response to binding and catalyzing ATP. Of course, they show different ATP-dependent transition regions from a non-cooperative motor to a cooperative motor. To further verify the conformation-correlation induced cooperativity, it will be worthwhile to combine the observations of kinetics/activities with structural biology analysis and molecular dynamics experiments. For example, in kinesin, a negative cooperativity has been structurally related to the binding of ATP to the empty head due to the release of ADP from the active head. Meanwhile, ATP-triggered detachment of the rear head may be an example of positive cooperativity [46]. For  $F_1$ -ATPase, Goffeau and coworkers [47] revealed a strong link between the nucleotide affinity and the negative cooperativity. They proposed a structural basis of cooperativity between the catalytic and non-catalytic subunits. Although the CC model reveals the mechanisms of cooperativity in kinesin, myosin and  $F_1$ -ATPase, the real structural and dynamic pathways behind the complex cooperativity, i.e., for the existence of both positive and negative cooperativity across a wide range of physiological conditions, remain hidden.

#### 4.3. From uni-site to multi-site: a steady operation of the ATP-fueled molecular motors

Strong cooperativity of activities intrinsically implies an interactive multiple site catalysis in molecular motors. To make this point clear, we take  $F_1$ -ATPase as an example. There has been disagreement in the literature [13] on the conformational binding-change schemes of  $F_1$ -ATPase, i.e., whether it is a ‘bi-site’ or ‘tri-site’ catalysis. The ultimate answer is currently obscured by the infeasibility of experimental determination of the binding states and their changes during steady hydrolysis. From the kinetics point of view, a ‘bi-site’ activity is the repetition of ‘uni-site’ catalysis [19,39], and no cooperativity occurs among different subunits. Although some experimental data indicates that [43] ‘bi-site’ catalysis is enough for a steady operation of  $F_1$ -ATPase, these results neglect the fact that two or more sites affect each other in the state and sequence of conformational changes, or even simultaneously carry out the same reaction pathway. Our simulations indicate that the binding states change as a function of [ATP] before the hydrolysis is saturated. The ‘bi-site’ activity, where no cooperativity between three catalytic subunits happens, is only valid for a limited region before saturation [19]. For a saturated steady operation,  $F_1$ -ATPase undergoes highly cooperative hydrolysis with three subunits affecting each other in catalysis activity due to conformational correlation at one time. This is consistent with other experimental observations [13,41,48] that the maximal activity was attained only when all catalytic sites were occupied. As a characteristic of ‘tri-site’ operation, the  $F_1$ -ATPase motor shows a cooperativity of either negative [18], positive [17] or complex combination [19]. Similarly, it should be expected that the steady operation of dimeric kinesin and myosin motors requires the two-subunit activity. Mostly, this induces cooperativity by the conformation correlation of different subunits.

It is of interest that, as shown in the dotted lines of Fig. 3 (A, B and C) and Fig. 4 (A, B and C), a transition in the mechanism comes about between null cooperativity (with  $h=1$ ) to full cooperativity. Due to this transition, the ATP-fueled molecular motors exhibit a negative cooperativity. An experiment with *Bacillus* PS3  $F_1$ -ATPase [18] showed that it is likely that the binding of ATP to the non-catalytic site activates the MgADP inhibition. Nonetheless, this is in conflict with the behavior observed in mitochondrial  $F_1$ -ATPase [17].

#### 4.4. To find the conformational correlation in a molecular motor

Our studies suggest that, for the steady operation of the multi-subunit molecular motors, cooperativity in motors is inevitable. However, this cooperativity has not been experimentally determined at the molecular level. A number of fundamental questions concerning the structural and

mechanochemical mechanisms of cooperativity remain unanswered. To name a couple, (1) communication between the different subunits is not clear, (2) a quantitative determination of conformational correlation is needed. To answer these questions, quantitative and dynamical experiments are urgently needed.

To investigate the possible conformational correlation in molecular motors, several experimental technologies can be used. These technologies may include, (1) single molecular biophysics, such as FRET [44,45], with focus on the dynamics and structural transitions, or (2) conformational dynamics studies by NMR and EPR spectroscopy [31]. For example, Karplus and Gao [49] have reviewed progresses on molecular biophysics of molecular motors. However, dynamic properties of structural transitions and conformational fluctuation in molecular motors have not been systemically and specifically researched. The present technologies are either limited by the size of proteins or by the spatial and dynamic resolutions. Further improvements of these technologies have to be done in order to reveal the conformational transitions and fluctuations, particularly in molecular motors. Recently, work has been attempted to probe the kinetics of uncorrelated reactions and enzymatic dynamics at the single molecule level [31,50 and references therein]. With more comprehensive atomistic structural and molecular dynamics investigations, the conformational correlations and its induced cooperativity shall be fully determined and understood in the multi-subunit molecular motors. Understanding of the cooperativity in molecular motors is a key to further manipulation and fabrication of molecular motors for purpose-design applications.

## 5. Conclusions

We have found that the motor activities of kinesin, myosin and  $F_1$ -ATPase have a complex ATP-dependent cooperativity, rather than following the simple Michaelis–Menten mechanism. Cooperativity of ATP catalysis appears to be a common characteristic of the ATP-fueled molecular motors. The cooperativity in kinetics and mechanochemics of molecular motors is shown to be induced by conformation correlation, namely by the interactive conformational coupling from different binding states of multiple subunits. Quantitative analysis and simulation indicate that conformation correlation prevails in the steady operations of the kinesin, myosin and  $F_1$ -ATPase molecular motors.

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