

# Coarse-grained modeling of conformational transitions underlying the processive stepping of myosin V dimer along filamentous actin

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#### ABSTRACT

To explore the structural basis of processive stepping of myosin V along filamentous actin, we have performed comprehensive modeling of its key conformational states and transitions with an unprecedented residue level of details. We have built structural models for a myosin V monomer complexed with filamentous actin at four biochemical states [adenosine diphosphate (ATP)-, adenosine diphosphate (ADP)-phosphate-, ADP-bound or nucleotide-free]. Then we have modeled a myosin V dimer (consisting of lead and rear head) at various two-head-bound states with nearly straight lever arms rotated by intramolecular strain. Next, we have performed transition pathway modeling to determine the most favorable sequence of transitions (namely, phosphate release at the lead head followed by ADP release at the rear head, while ADP release at the lead head is inhibited), which underlie the kinetic coordination between the two heads. Finally, we have used transition pathway modeling to reveal the order of structural changes during three key biochemical transitions (phosphate release at the lead head, ADP release and ATP binding at the rear head), which shed lights on the strain-dependence of the allosterically coupled motions at various stages of myosin V's work cycle. Our modeling results are in agreement with and offer structural insights to many results of kinetic, single-molecule and structural studies of myosin V.

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Key words: myosin V; coarse-grained model; elastic network model; transition pathway; kinetic coordination; strain; lever arm; processive stepping; ADP release; ATP binding.

### INTRODUCTION

Myosins are a superfamily of actin-based motor proteins powered by the hydrolysis of adenosine triphosphate (ATP). They are involved in a variety of functions ranging from muscle contraction to intracellular transportation.<sup>1</sup> Among more than 20 myosin classes,<sup>2</sup> class II myosin has been extensively investigated for decades by biochemical, biophysical, genetic, and structural studies (for reviews see Refs. 3–5). In the past decade, tremendous interests have been attracted by the class V myosin (myosin V)—a dimeric motor that walks along filamentous actin (F-actin) processively by alternating its two heads in a hand-overhand fashion (for reviews see Refs. 6–10).

The primary kinetic cycle of a monomeric myosin, which is conserved among various myosin classes, has been outlined by extensive kinetic studies<sup>11-13</sup>:

# $M-ATP \rightarrow M-ADP-Pi \rightarrow A-M-ADP-Pi \rightarrow$ $A-M-ADP \rightarrow A-M \rightarrow A-M-ATP \rightarrow M-ATP,$ (A + actin M + mussin M

(A: actin, M: myosin, Pi:  $\gamma$ -phosphate)

Starting from M-ATP state (postrigor state), ATP hydrolysis leads to M-ADP-Pi state (pre-powerstroke state), which is accompanied by an upward rotation of the lever arm to the pre-powerstroke position (recovery stroke). Actin binding accelerates Pi release from myosin and leads to A-M-ADP state, resulting in force generation (powerstroke) as the lever arm rotates downward to the post-powerstroke position.<sup>3–5</sup> Subsequent release of ADP leads to A-M state, which is accompanied by a further downward rotation of the lever arm in some myosins including myosin V.<sup>14–18</sup> ATP binding dissociates myosin rapidly from actin<sup>19</sup> and returns it for the next cycle. In myosin V, ADP release (or the isomerization from strong to weak ADPbinding state prior to ADP release) is rate limiting.<sup>19,20</sup> Therefore,

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the kinetic cycle time of myosin V is dominated by strong actin-binding states, which enables it to walk along F-actin for many steps before dissociation.<sup>19</sup>

In a myosin V dimer composed of two heads (lead head and rear head), both heads undergo transitions following the above kinetic cycle. The kinetics of the two heads must be coordinated so that at least one head is strongly bound with F-actin at any time. Such kinetic coordination is required for the processive stepping of myosin V along F-actin. However, the mechanism of kinetic coordination remains controversial. In one scenario, it is postulated that Pi is rapidly released from the lead head on binding with F-actin while ADP release from the lead head is inhibited by the backward pulling force.<sup>21-24</sup> In another scenario, Pi release from the lead head is hindered until the rear head detaches from F-actin.<sup>13</sup> It was also suggested that multiple kinetic pathways may coexist for myosin V dimer, which are populated under different conditions.<sup>25–27</sup>

The kinetics of monomeric and dimeric myosin are thought to involve a cascade of nucleotide-dependent conformational transitions among a series of biochemical states—these conformational transitions may orchestrate the allosteric couplings among actin binding/release, nucleotide binding/release and force generation within each myosin head, and the kinetic coordination between two heads. To understand the structural basis of myosin motor function, it is critical to probe these biochemical states and conformational transitions with high spatial and temporal resolutions.

Thanks to decades of structural studies of myosins, detailed structural information is available for some but not all biochemical states of actomyosin. High-resolution myosin structures bound with various nucleotide analogs were solved by X-ray crystallography for two weak actinbinding states (M-ATP and M-ADP-Pi state; refs. 28-32) and a rigor-like state that allows strong binding with F-actin.<sup>33-36</sup> To date, no crystal structure of actomyosin at strong actin-binding states has been solved. Low-resolution actomyosin models were built by docking myosin crystal structure and F-actin model<sup>37</sup> into cryo-electron microscopy (cryo-EM) maps of myosin-decorated actin filaments.<sup>16,38–42</sup> The docking studies have revealed a closed actin-binding cleft in a nucleotide-free or ADPbound myosin motor domain.<sup>16,38,41</sup> The lack of detailed structural information for strong actin-binding states (particularly A-M-ADP state) has hindered a complete understanding of the structural events that couple actin binding to the release of hydrolysis products and force generation in each myosin head and coordinate the kinetics of two heads.

The highly conserved myosin motor domain consists of four subdomains—upper and lower 50 kDa subdomains (U50 and L50), N-terminal subdomain and converter subdomain (Fig. 1). The nucleotide-binding site, located at the interface between U50, L50 and N-terminal



#### Figure 1

Detailed view of structural differences between various biochemical states of myosin V (**A-M-ADP-Pi**: blue, **A-M-ADP**: green, **A-M**: red, **A-M-ATP**: yellow). Key structural elements involved in actin binding (HLH motif, CM loop), nucleotide binding (P loop, switch I, switch II), and force generation (relay helix, lever arm) are highlighted and labeled. The actin subunits in contact with myosin are colored black while the rest of F-actin is in gray. The dotted lines outline the four subdomains—upper and lower 50 kDa (U50 and L50), N-terminal (N), and converter (C), which are connected by several flexible joints (such as relay helix and switch II).

subdomains, consists of three conserved motifs essential for nucleotide binding/hydrolysis—P loop, switch I, and switch II (Fig. 1). The outer cleft between U50 and L50 subdomains is involved in actin binding.<sup>38</sup> The converter is adjoined to a lever arm which generates force via a large rotation.<sup>3–5</sup> In myosin V, the lever arm consists of a long  $\alpha$ -helix with six IQ motifs for binding six light chains. It is believed that the above myosin parts are allosterically coupled via several flexible joints<sup>32</sup> (such as switch II, relay helix and SH1 helix, see Fig. 1) and a central  $\beta$ -sheet that spans from U50 to N-terminal sub-domain.<sup>34–36</sup>

Structure-based computer simulation has been widely used to elucidate the structural origins of myosin motor function. At atomic resolution, molecular dynamics (MD) simulation, Brownian dynamics simulation, energy minimization, and quantum mechanical calculation have been used to probe active-site dynamics of myosin,<sup>43,44</sup> ATP hydrolysis,<sup>45–49</sup> Pi release,<sup>50</sup> recovery stroke,<sup>51–57</sup> ATP binding,<sup>58,59</sup> elastic properties,<sup>60</sup> and actin–myosin interactions.<sup>61,62</sup> However, the exceedingly long time scale of myosin kinetic cycle (up to  $\sim 10^{-1}$  s) has hindered extensive MD simulations of conformational transitions between the biochemical states of actomyosin.

To surmount the timescale barrier, coarse-grained models have been developed using simplified structural representations and energy functions.<sup>63,64</sup> A good example of coarse-grained models is the elastic network model (ENM), which represents a protein structure as a network of  $C_{\alpha}$  atoms connected by springs<sup>65–67</sup> with a uniform force constant<sup>68</sup> (or two different force constants<sup>69</sup> for bonded and nonbonded residue pairs, see Methods section). Early studies have demonstrated that the collective motions predicted by the normal mode analysis (NMA) of ENM are highly robust to the simplification in ENM.<sup>65–67</sup> The low-frequency modes calculated from ENM were found to compare well with many crystallographically observed structural changes.<sup>67,70</sup> Numerous studies have established ENM as an efficient means to probe protein conformational dynamics with virtually no limit in timescale or system size (for reviews see refs. 71-73). Recently, the NMA based on ENM or all-atom force fields has been used to study global motions of myosin,74-76 local motion at the nucleotide-binding site and its coupling with global motions,<sup>77</sup> dynamic couplings,<sup>52,78-80</sup> structural flexibility,81 conformational transition pathway,82-84 and regulation.85 These studies have demonstrated the usefulness of ENM and coarse-grained modeling in probing myosin's conformational dynamics.

In a recent study,<sup>86</sup> by combining coarse-grained modeling of global conformational changes and atomistic simulation of active-site dynamics, we have investigated how actin binding triggers distant structural changes that result in the release of hydrolysis products and force generation in a myosin motor domain. Our modeling of allosteric couplings has identified key actin-activated couplings critical to force generation and the sequential release of Pi and ADP, and isoform-dependent couplings underlying the reciprocal coupling<sup>87–90</sup> between actin binding and nucleotide binding in fast myosin II, and load-dependent ADP release<sup>24</sup> in myosin V. Our transition pathway modeling has predicted that the mechanical movements (rotation of converter and lever arm) start immediately on actin binding, which are followed by the twisting of central  $\beta$ -sheet and later the opening of nucleotide-binding site to allow ADP release. Our atomistic simulation of active-site dynamics has revealed significantly weakened coordination of Pi by switch II and a disrupted key salt bridge between switch I and switch II, while the coordination of MgADP by switch I and P loop is less perturbed. It will be interesting to extend our coarse-grained modeling to myosin V dimer to further understand the effect of intramolecular strain on allosterically coupled motions within each myosin head and kinetic coordination between two heads.

In this study, we will explore the structural basis of processive stepping of myosin V dimer by modeling its

key conformational states and transitions with a residue level of details. First, we will build structural models of a myosin V monomer bound with F-actin at four biochemical states (A-M-ATP, A-M-ADP-Pi, A-M-ADP, and A-M state), which will reveal key structural changes involved in force generation and the binding/release of actin, Pi, and ADP. Second, we will build structural models of a myosin V dimer at various two-head-bound states which feature nearly straight lever arms rotated by strain. Third, we will perform transition pathway modeling to determine the most favorable sequence of transitions (Pi release at the lead head followed by ADP release at the rear head, while ADP release at the lead head is inhibited), which underlie the kinetic coordination in myosin V. Fourth, we will use transition pathway modeling to reveal the order of structural changes during three biochemical transitions (Pi release at the lead head, ADP release, and ATP binding at the rear head) in the presence of strain. Our modeling will offer rich structural insights to many results of previous kinetic, single-molecule, and structural studies of myosin V.

### METHODS

#### F-actin model

We use an atomic model of F-actin obtained from the fitting of X-ray fibre diagrams.<sup>37</sup> The F-actin model consists of 18 actin subunits, where two subunits are in contact with the lead (rear) head of myosin V dimer [Fig. 2(a)]. To reduce computing cost, we model the flexibility of F-actin by allowing residues of the myosin-contacting actin subunits to move while fixing the rest of F-actin (the residue coordinates of the fixed actin subunits are kept constant during the structural displacements along the transition pathways, see below). Alternatively, the full dynamics of F-actin was previously explored experimentally.<sup>91</sup> and computationally.<sup>92</sup>

#### Monomeric actomyosin model

We model the following biochemical states of a myosin V monomer (truncated at residue 909) bound with F-actin [see Fig 2(a)].

#### A-M state

It was constructed by Holmes et al.<sup>37,42</sup> who fitted the crystal structure of a nucleotide-free myosin V motor domain (PDB: 1W8J) and an atomic model of F-actin into a cryo-EM map of myosin-decorated F-actin. We then fuse an atomic model of myosin V lever arm bound with six light chains<sup>93</sup> with the myosin V motor domain by superimposing them along the N-terminal region of lever arm (residues 755–764). Same fusion is done for **A-M-ATP** and **A-M-ADP-Pi** state (see below).



#### Figure 2

Global view of structural models of lead head and rear head of myosin V bound with F-actin. (a) The two heads are docked on F-actin separately without dimerization and colored by biochemical state (A-M-ADP-Pi: blue, A-M-ADP: green, A-M: red, A-M-ATP: yellow), the four actin subunits in contact with myosin are colored black while the rest of F-actin is in gray; the arrows show the forward displacements of lever-arm tip (residue 909) accompanying Pi release and ADP release, and the rotation of lever arm accompanying ADP release. The inset on the right shows off-axis rotations of lever arm (viewed along F-actin axis) accompanying Pi and ADP release (residue 909 is shown as a sphere). The model colored in cyan is an intermediate conformation during the transition from A-M-ADP-Pi state to A-M-ADP state. (b) The two-heads are dimerized by enforcing a distance constraint for residues 909 at the tip of two lever arms (shown as two spheres connected by a short line), four two-head-bound states are shown and colored as follows—(A-M-ADP, A-M-ADP-Pi): blue, (A-M-ADP, A-M-ADP): green, (A-M, A-M-ADP): red, and (A-M-ATP, A-M-ADP): yellow; the arrows show the on-axis and off-axis displacements of lever-arm tip (residue 909) between (A-M-ADP, A-M-ADP) state and the strain-free A-M-ADP state of lead and rear head.

#### A-M-ATP state

It is constructed from the crystal structure of an ATP analog-bound myosin V motor domain (PDB: 1W7J). This structure is docked on F-actin by superimposing it with the **A-M** state model along the HLH motif of L50 subdomain (residues 492–533, see Fig. 1; following Ref. 42). Same docking is done for **A-M-ADP-Pi** state (see below).

#### A-M-ADP-Pi state

For the lack of a pre-powerstroke crystal structure of myosin V, we use a crystal structure of *Dictyostelium* my-

osin II (PDB: 1VOM) as template to build a homology model of myosin V with Swiss Model Server.<sup>94</sup> However, due to low sequence similarity in the N-terminal subdomain and missing residues in the converter of 1VOM, these two subdomains cannot be completely modeled by homology modeling. To build a complete model of myosin V motor domain from the incomplete homology model based on 1VOM, we use the interpolated ENM (iENM)<sup>84</sup> to interpolate from a nucleotide-free myosin V structure (PDB: 1W8J), whose N-terminal and converter subdomains are fully resolved, to the 1VOMbased homology model with truncated N-terminal and converter subdomains. The interpolation procedure essentially fuses the N-terminal and converter subdomains of 1W8J to the 1VOM-based homology model (see Supporting Information Fig. S1).

To validate the above modeling procedure, we use the iENM protocol to interpolate from a nucleotide-free structure of *Dictyostelium* myosin II (PDB: 2AKA, residues 2–765), whose N-terminal and converter subdomains are fully resolved, to the incomplete structure of 1VOM with truncated N-terminal (residues 2–98) and converter (residues 708–747) subdomains. Then we compare the interpolated model with the complete structure of 1VOM (residues 2–747) and obtain a root mean squared deviation (RMSD) of ~1 Å. Therefore, the iENM-based interpolation can accurately generate a complete structure of myosin motor domain from an incomplete structure with truncated N-terminal and converter subdomains.

#### A-M-ADP state

We use the iENM protocol<sup>84</sup> to generate a pathway (i.e., a series of intermediate conformations) from the **A-M-ADP-Pi** state model to the **A-M** state model. We then select an intermediate conformation of the pathway, which satisfies the constraint that the lever arm is rotated forward by  $\sim 22^{\circ}$  from **A-M-ADP** state to **A-M** state [see Fig. 2(a)]. This constraint is derived from a real-time atomic force microscopy (AFM) study of ADP-bound single-headed myosin V, which found that the orientational angle of lever arm (relative to F-actin axis) follows a double-Gaussian distribution peaked at  $\sim 29^{\circ}$  and  $\sim 51^{\circ}.95$ 

#### Dimeric actomyosin model

Two myosin V heads (L: lead head, R: rear head) are docked on F-actin with a separation of 13 actin subunits [see Fig. 2(a)]. Each head is at A-M-ATP, A-M-ADP-Pi, A-M-ADP, or A-M state. The two heads are dimerized by connecting the  $C_{\alpha}$  atoms of residues 909 (at the tip of lever arms) with a spring of equilibrium length 3.5 nm [see Fig. 2(b)]. This distance information is obtained from an EM-fitted model of myosin V dimer (PDB: 2DFS).<sup>96</sup> Then we run energy minimization to generate dimeric models of myosin V, which results in slight bending and small/large rotation of the lever arm of rear/ lead head [see Fig 2(b)].

#### Elastic network model of actomyosin

By using an ENM, we represent an actomyosin structure as a network of beads each corresponding to the  $C_{\alpha}$ atom of an amino acid residue. A harmonic potential accounts for the interaction between a pair of  $C_{\alpha}$  atoms that are within a cutoff distance  $R_c$ . Following our earlier study,<sup>86</sup>  $R_c$  is set to 10 Å for all pairs of residues except those within the lever arm and converter (see below). An optimal  $R_c = 10$  Å was also obtained by the ENM-based modeling of anisotropic atomic fluctuations in protein crystals.<sup>97,98</sup> The ENM potential energy is

$$E = \frac{1}{2} \sum_{i=2}^{N} \sum_{j=1}^{i-1} k_{ij} \theta(R_{\rm c} - d_{ij,0}) (d_{ij} - d_{ij,0})^2, \qquad (1)$$

where *N* is the number of residues,  $\theta(x)$  is the Heaviside function,  $d_{ij}$  is the distance between the  $C_{\alpha}$  atom *i* and *j*,  $d_{ij,0}$  is the value of  $d_{ij}$  as given by an equilibrium structure.  $k_{ij}$  is the force constant which is set to  $k_0$  for nonbonded interactions and  $10k_0$  for bonded interactions between residues. Here,  $k_0$  is determined by fitting the crystallographic B factors of a myosin V crystal structure (PDB: 1W8J).<sup>99</sup> The fitting gives  $k_0 = 0.3k_{\rm B}T/\text{Å}$ ,<sup>2</sup> where  $k_{\rm B}$  is the Boltzmann constant, and T = 300 K is room temperature. The use of high/low force constant for bonded/nonbonded interactions was previously found to improve the accuracy of ENM-based modeling.<sup>69</sup>

For an ENM, the Hessian matrix H is calculated as the second derivatives of potential energy E [see Eq. (1)] with respective to  $C_{\alpha}$  coordinates. The mean-squared fluctuation of  $C_{\alpha}$  atom *i* can be calculated from the Hessian matrix:

$$\left< \delta r_i^2 \right> = k_{\rm B} T \cdot \text{trace}[H_{ii}^{-1}]$$
 (2)

where  $H_{ii}^{-1}$  is the *i*th diagonal super-element of the inverse of Hessian matrix.

We assume that the lever arm and converter together form a stiff mechanical unit, which is represented by an ENM with a high cutoff distance ( $R_c = 40$  Å, determined below). Such assumption is necessary to explain the EM observations that the converter and lever arm both assume the pre-powerstroke position in the lead head of myosin V, and the lever arm is nearly straight despite strain.<sup>100,101</sup> Otherwise, if the lever arm and converter are assumed to be as flexible as the motor domain (with  $R_c = 10$  Å), the resulting lead-head model would have a strongly bent lever arm; or, if only the lever arm is assumed to be stiff (with  $R_c = 40$  Å), the resulting leadhead model would have a converter in the post-powerstroke position and a lever arm tilted backward.

In a previous mechanochemical modeling of myosin V, the stiffness of lever arm (~1500 pN × nm<sup>2</sup>, corresponding to a persistent length  $L_{\rm p} \sim 375$ nm) was estimated based on the fitting of step size of myosin V under load.<sup>102</sup> We fit the above persistent length by choosing  $R_{\rm c} = 40$  Å for pairs of residues within the lever arm and converter. To this end, the persistent length is calculated as follows<sup>103</sup>:

$$L_{\rm p} \sim \frac{2L}{3\langle \delta r_{\perp}^2 \rangle} \tag{3}$$

where  $L \sim 24$  nm is the length of lever arm, and  $\langle \delta r_{\perp}^2 \rangle$  is the transverse mean-squared fluctuation of the tip of lever arm (residue 909) perpendicular to the lever arm (assuming

F-actin is fixed).  $\langle \delta r_{\perp}^2 \rangle$  can be computed directly using Eq. (2) because the longitudinal fluctuation of lever arm is much smaller than its transverse fluctuation.<sup>102</sup>

#### Transition pathway modeling by iENM

To generate a pathway (i.e., a series of intermediate conformations) between two given protein conformations (named beginning and end conformation), we first construct two single-well potentials ( $E_1$  and  $E_2$ ) whose minima are located at the two given conformations, respectively. Then we construct a double-well potential  $F(E_1, E_2)$  with two minima located at the two given conformations. Then we solve the saddle points (SP) of  $F(E_1, E_2)$  as follows:

$$0 = \nabla F(E_1, E_2) = \frac{\partial F}{\partial E_1} \nabla E_1 + \frac{\partial F}{\partial E_2} \nabla E_2, \qquad (4)$$

which is equivalent to solving the following equation (after setting  $\lambda = (\partial F / \partial E_1) / (\frac{\partial F}{\partial E_1} + \frac{\partial F}{\partial E_2})$ )

$$0 = \lambda \nabla E_1 + (1 - \lambda) \nabla E_2, \tag{5}$$

where  $\lambda$  is a parameter of interpolation—as  $\lambda$  varies from 1 to 0, the SP traces a pathway that connects the beginning and end conformation. Because this pathway passes all possible SPs, it is independent of the mathematic form of  $F(E_1, E_2)$ .<sup>84</sup>

Following the above general formulation, we have proposed an iENM protocol<sup>84</sup> which solves the SPs of a double-well potential  $F(E_{\text{ENM1}} + E_{\text{col}}, E_{\text{ENM2}} + E_{\text{col}})$ , where  $E_{\text{ENM1}}$  and  $E_{\text{ENM2}}$  are two ENM potentials [see Eq. (1)] based on the beginning and end conformations, and  $E_{\text{col}}$  is the steric collision energy.<sup>84</sup> The iENM method does not require an initial guess of the pathway, and it uniquely generates a single pathway between the two given conformations.

# **Q**uantification of motional order of key myosin parts during a transition

Following Ref. 104, a fractional progress parameter  $f_{\text{progress}}$  ( $f_{\text{progress}} \in [0,1]$ ) is defined for an intermediate conformation along a transition pathway:  $f_{\text{progress}} = l/L$ , where l is the length of the part of the pathway from the beginning conformation to the intermediate conformation and L is the total length of the pathway from the beginning conformation to the end conformation. The length of a pathway is computed approximately by summing up RMSDs between consecutive conformations along the pathway (the pathway is sample at a step size of 0.1 Å in RMSD).

The predicted pathway allows us to determine the motional order of two parts of myosin. To this end, the following reaction coordinate is defined for a given part  $S^{86}$ :

$$RC_{S} = 0.5 \left( 1 + \frac{RMSD_{S,1}^{2} - RMSD_{S,2}^{2}}{RMSD_{S,obs}^{2}} \right),$$
 (6)

where  $\text{RMSD}_{S,1}(\text{RMSD}_{S,2})$  is the RMSD of  $C_{\alpha}$  atoms of part S between a given intermediate conformation and the beginning (end) conformation, and  $\text{RMSD}_{S,\text{obs}}$  is the corresponding RMSD between the beginning and end conformation. RC<sub>S</sub> varies from 0 to 1 as the transition proceeds from the beginning to the end conformation. For two myosin parts S and S', if RC<sub>S</sub> < RC<sub>S'</sub> along the pathway, we can infer that the motion of S' precedes that of S.

## **RESULTS AND DISCUSSION**

#### Modeling of monomeric actomyosin V

Our structural modeling of monomeric actomyosin V is based on the synthesis of crystal structures of myosin V and myosin II, a cryo-EM-fitted model of monomeric actomyosin V at rigor state,<sup>42</sup> an atomic model of F-actin,<sup>37</sup> and an atomic model of myosin V lever arm bound with six light chains.<sup>93</sup> At a residue level of details, we have modeled myosin V monomer bound with F-actin at four biochemical states (A-M-ADP-Pi, A-M-ADP, A-M, and A-M-ATP state; see Methods section). The structurally unknown A-M-ADP state is modeled by interpolating between the A-M-ADP-Pi state model and the A-M state model using a transition pathway modeling protocol named iENM<sup>84</sup> (see Methods section).

A detailed comparison of the above models has offered structural insights to the biochemical and mechanical function of myosin V:

- At the actin-binding interface, the L50 subdomain is anchored on F-actin in all four models. The actinbinding cleft is open at A-M-ADP-Pi and A-M-ATP state, partially closed at A-M-ADP state and further closed at A-M state (see Fig. 1). These structural changes underlie low actin-binding affinity of myosin V at A-M-ADP-Pi and A-M-ATP states, intermediate and high actin-binding affinities at A-M-ADP and A-M states, respectively.<sup>19</sup> In agreement with our finding, a cryo-EM study of smooth muscle myosin found that the ADP-bound state has a partially closed cleft that closes further on nucleotide release,<sup>40</sup> a mechanical study found a lower unbinding force of actomyosin V in the presence of ADP than in the absence of nucleotide.<sup>105</sup>
- 2. At the nucleotide-binding site, switch II is well superimposed in all four models. At A-M-ADP and A-M states, switch I adopts a similar "up" position relative to switch II (see Fig. 1). Such switch I–II conformation may enable the release of Pi through a "backdoor" between the two switches.<sup>86,106</sup> At A-M-ADP-Pi and A-M-ATP states, switch I adopts different

"down" positions relative to switch II (see Fig. 1). Such local structural change following hydrolysis may propagate via relay helix to converter and lead to recovery stroke.<sup>51,84</sup> Relative to switch I, P loop adopts an open conformation at **A-M** state, a closed conformation at **A-M-ADP-Pi** state, and a half-closed conformation at **A-M-ADP** state (see Fig. 1). This is consistent with the finding of a dynamic equilibrium between open and closed switch I conformation (relative to P loop) in the presence of ADP,<sup>107,108</sup> and a decrease in ADP-binding affinity from **A-M-ADP** state to **A-M** state (because ADP is primarily coordinated by switch I and P loop).

3. The converter and lever arm adopt an upward/backward position with kinked relay helix at A-M-ADP-Pi state, a downward/forward position with straight relay helix at A-M state, and an intermediate position at A-M-ADP state [see Figs. 1 and 2(a)]. As a result, the lever arm swings forward by  $\sim 50^{\circ}$  during Pi release and  $\sim 22^{\circ}$  during ADP release; the lever-arm tip (residue 909) moves forward along F-actin axis by  $\sim$ 19 nm during Pi release and  $\sim 8$  nm during ADP release [see Fig. 2(a)]. Our modeling results agree with the 20-25 nm work stroke of myosin V found by mechanical measurements,18,109 which comprised two phases associated with Pi release and ADP release.<sup>18</sup> Our finding of  $\sim$ 8nm displacement during ADP release, although higher that the 5-nm displacement associated with ADP release in a mechanical study,<sup>18</sup> is compatible with the distance parameter ~6.5 nm for the load dependence of kinetics of ADP release.<sup>110</sup> Unlike our results, a cryo-EM study<sup>16</sup> found a small lever-arm rotation of  $\sim 6^{\circ}$  (or a displacement of 2.4 nm) associated with ADP release. It is likely that the cryo-EM study<sup>16</sup> captured another ADP state with strong affinity for actin and weak affinity for ADP, which is structurally closer to the rigor state.<sup>35</sup>

Further analysis of the transition pathway from A-M-ADP-Pi state to A-M-ADP state reveals an off-axis rotation of the lever arm early during the transition [ $\sim 12^{\circ}$  around the F-actin axis, see Fig. 2(a)] Therefore, the transition from A-M-ADP-Pi state to A-M-ADP state is coupled to lever-arm rotation with both an on-axis and an off-axis component. This result explains the finding that the actomyosin bond is weakened if an off-axis backward load (around  $+20^{\circ}$ ) is applied to a single-headed myosin V,<sup>105</sup> which opposes the above on-axis and off-axis rotation of lever arm and thus reverses the transition. We have also found a smaller off-axis rotation ( $\sim 4^{\circ}$ ) of lever arm during the transition from A-M-ADP state to A-M state [see Fig. 2(a)], which may enable the modulation of ADP release by off-axis strain.<sup>23,105</sup>

#### Modeling of dimeric actomyosin V

By using the above monomeric actomyosin V models, we have further modeled a dimeric actomyosin V at various two-head-bound states denoted as  $(S_R, S_L)$ , where  $S_R/S_L$  represents the biochemical state of the rear/lead head  $(S_R/S_L = A-M-ADP-Pi, A-M-ATP, A-M-ADP, or A-M state)$ . To dimerize two myosin heads, we impose a distance constraint for the lever-arm tips of two myosin monomers docked on F-actin with a separation of 13 actin subunits [see Methods section and Fig. 2(b)]. The satisfaction of this distance constraint introduces intra-molecular strain, which causes the lever arm of the lead/ rear head to rotate in the backward/forward direction.

Among all 16 possible two-head-bound states, we will focus on the following four states [see Fig. 2(b)], which constitute the most favorable kinetic path of myosin V dimer (see next subsection).

#### (A-M-ADP, A-M-ADP-Pi) state

The dimerization causes the lead-head lever arm to rotate backward by  $\sim 16^{\circ}$  and the rear-head lever arm to rotate forward by  $\sim 23^{\circ}$ . Both lever arms remain nearly straight [see Fig. 2(b)]. This state is reached following a thermally driven diffusive search of the lead head (bound with ADP and Pi) for the next actin-binding site.<sup>111,112</sup> Because the lead/rear-head lever arm is at pre-/postpowerstroke position prior to dimerization, the dimerization only induces weak strain which does not cause significant changes to the lead and rear head. As Pi release is fast,<sup>19</sup> this state is only transiently dwelled.

#### (A-M-ADP, A-M-ADP) state

The dimerization causes the lead-head lever arm to tilt strongly backward by  $\sim 60^{\circ}$  and the rear-head lever arm to rotate moderately forward by  $\sim 30^{\circ}$ . As a result, the angular position of the lead-head lever arm ( $\sim 130^{\circ}$  relative to F-actin axis) is indistinguishable from (A-M-ADP, A-M-ADP-Pi) state-both in the pre-powerstroke position [see Fig. 2(b)]. Both lever arms remain nearly straight although the lead-head lever arm is slightly bent backward [see Fig. 2(b)]. The backward strain causes the lead-head converter to rotate backward so the lever arm emerges from an intermediate position between A-M-ADP-Pi and A-M-ADP states [see Fig. 2(b)]. However, the forward strain causes little change to the rear head so the lever arm still emerges from the same position as **A-M-ADP** state [see Fig. 2(b)]. The tips of both lever arms are moved forward by only  $\sim 2$  nm relative to (A-M-ADP, A-M-ADP-Pi) state, which is much smaller than the ~19-nm displacement of lever-arm tip associated with Pi release in monomeric actomyosin V [see Fig. 2(a)]. Therefore, the powerstroke accompanying Pi release at lead head cannot be completed mechanically. Instead, it stores energy as strain which is released later to drive large displacement of myosin V dimer along F-actin following the detachment of rear head.

As ADP release is rate limiting,<sup>19</sup> this state is dominantly populated. At this state, the angle of lever arm relative to F-actin axis is ~42° for the rear head and ~131° for the lead head, which agrees well with the angles (~40° for the rear head and 130–145° for the lead head) determined by EM studies.<sup>100,113</sup> Our modeling predicts that the angular difference between the lead-head and rear-head lever arm is ~90°, which is higher than the value (71° or 75°) measured by single-molecule fluorescence imaging.<sup>114,115</sup> This discrepancy may be attributed to deviation between the angles of fluorescence probes and lever arms caused by positional uncertainty of probes on lever arms and slight bending of lever arms.

#### (A-M, A-M-ADP) state

The dimerization causes the lead-head lever arm to tilt strongly backward by  $\sim 60^{\circ}$  and the rear-head lever arm to rotate slightly forward by  $\sim 10^{\circ}$ . The angular positions of both lever arms are similar to (A-M-ADP, A-M-ADP) state [see Fig. 2(b)]. So ADP release from rear head is accompanied by little displacement ( $\sim 0.7$  nm) of the lever-arm tips [see Fig. 2(b)], which is in contrast to the  $\sim$ 8-nm displacement of lever-arm tip associated with ADP release in monomeric actomyosin V. This result is in agreement with the finding of weak force dependence of stepping velocity at low backward forces, 27,116,117 which is limited by ADP release under saturating ADP concentration. A previous mechanochemical modeling<sup>102</sup> also found little movement at the center of mass of myosin V dimer following internal conformational changes within each head. Based on our finding, we infer that the 5-nm substep observed by single-molecule experi-ments<sup>17,18,118</sup> is not associated with ADP release.

#### (A-M-ATP, A-M-ADP) state

The angular positions of both lever arms are similar to (A-M, A-M-ADP) state [see Fig. 2(b)]. So ATP binding to rear head is not accompanied by displacement of the lever-arm tips. As the detachment of ATP-bound myosin V from F-actin is fast,<sup>19</sup> this state is only transiently dwelled by actomyosin V before transiting to a one head-bound state with ADP bound to the attached head and ATP hydrolysis occurring in the detached head.

Taken together, our structural modeling of two-headbound states predicts little motion of myosin V dimer as it undergoes the following transitions: (A-M-ADP, A-M-ADP-Pi)  $\rightarrow$  (A-M-ADP, A-M-ADP)  $\rightarrow$  (A-M, A-M-ADP)  $\rightarrow$  (A-M-ATP, A-M-ADP). Large displacements of myosin V dimer only happen when the rear head detaches and completes a strain-driven powerstroke (phase I) and then undergoes a diffusive search for the next actin-binding site (phase II, see Refs. 111,112). These displacements are mechanically and thermally driven and occur spontaneously as observed in a recent AFM study.<sup>95</sup> By comparing (A-M-ADP, A-M-ADP) state and the A-M-ADP state of unstrained lead (rear) head, we have found the lever-arm tip moves by ~22 nm (~10nm) along F-actin during phase I (II) [see Fig. 2(b)]. The total displacement of ~32 nm is a little less than the 36-nm step because of the 3.5 nm gap between the two lever-arm tips [see Fig. 2(b)]. Accompanying the above on-axis displacements are ~5.5 nm off-axis motions away from (during phase I) and toward (during phase II) F-actin [see Fig. 2(b)]. Our finding agrees well with the results of mechanical measurements that the 36 nm step of myosin V dimer is composed of a working stroke of 23–25 nm and a diffusive substep of ~11 nm, 17,18,27,118 and the former is accompanied by a vertical motion of 6 nm away from F-actin. <sup>118</sup>

The dimerization-induced strain causes the lever arm of lead/rear head to rotate relative to the monomeric states. In particular, the ADP-bound lead head tilts its lever arm strongly backward, which is achieved by a combination of backward rotation of converter and slight bending of lever arm. Our modeling suggests that the strongly curved "telemark" conformation of lead-head lever arm <sup>113</sup> is energetically unfavorable, and the lever arm of lead head remains nearly straight despite the strain. This is in agreement with recent EM and fluorescence imaging studies.<sup>100,101,115,119</sup>

# Probing kinetic coordination by transition pathway modeling

Multiple kinetic pathways were proposed for myosin V.25-27 It remains controversial which pathway is most populated by myosin V dimer in support of its processive walking. To address this critical issue, we will determine the most favorable sequence of transitions through the two-head-bound states of actomyosin V as constructed above [see Fig. 2(b)], which underscores the kinetic coordination between lead and rear head. We will use the following procedure. Assuming that a myosin V dimer is at state  $(S_{R1}, S_{L1})$   $(S_{R1}/S_{L1}$  represents the biochemical state of rear/lead head), and the next biochemical state following  $S_{R1}/S_{L1}$  is  $S_{R2}/S_{L2}$ , we will determine whether the transition at lead head (S<sub>L1</sub>  $\rightarrow$  S<sub>L2</sub>) or rear head (S<sub>R1</sub>  $\rightarrow$ S<sub>R2</sub>) is energetically favored. To this end, we will use the iENM protocol<sup>84</sup> to generate a pathway of intermediate conformations from  $(S_{R1}, S_{L1})$  state to  $(S_{R2}, S_{L2})$  state and then analyze the relative order of the structural changes within rear head  $(S_{\rm R1} \rightarrow S_{\rm R2})$  and lead head  $(S_{\rm L1}$  $\rightarrow$  S<sub>L2</sub>) (excluding converter and lever arm, see Methods section): if  $S_{R1} \rightarrow S_{R2}$  precedes  $S_{L1} \rightarrow S_{L2}$ , then we predict that the transition at rear head  $(S_{R1} \rightarrow S_{R2})$  is favored, resulting in  $(S_{R2}, S_{L1})$  state; otherwise, we predict that the transition at lead head  $(S_{L1} \rightarrow S_{L2})$  is favored, resulting in  $(S_{R1}, S_{L2})$  state. The iENM protocol has been successfully used to predict order of structural events in various protein complexes including myosin motor domain.84,86,120

First, we start from (A-M-ADP, A-M-ADP-Pi) state and determine whether Pi release at lead head or ADP



#### Figure 3

Results of reaction coordinate (RC) analysis of iENM-generated pathways for: (a). transition from (A-M-ADP, A-M-ADP-Pi) to (A-M, A-M-ADP) state; (b) transition from (A-M-ADP, A-M-ADP, A-M-ADP) to (A-M, A-M) state; (c) transition from (A-M-ADP, A-M-ADP, A-M-ADP) to (A-M-ADP, A-M-ADP) state; (d) transition from (A-M-ADP, A-M-ADP) to (A-M, A-M-ADP) state; (e) transition from (A-M-ADP, A-M-ADP) to (A-M-ADP, A-M-ADP) state; (a) and (b), the red/blue curve corresponds to RC of rear/lead head; in (c)–(e), the red, blue, and green curve corresponds to RC of acin-binding cleft, converter and L50, and nucleotide-binding site; the solid/dashed curve corresponds to the pathway generated in the presence/absence of strain. Note that RC does not always start at 0 or end at 1 because of strain-induced conformational changes to the beginning/end conformations of the above transitions.

release at rear head occurs next. We generate a transition pathway from (A-M-ADP, A-M-ADP-Pi) state to (A-M, A-M-ADP) state and then analyze the reaction coordinates for lead and rear head. We find the reaction coordinate of lead head is higher than that of rear head along most of the transition pathway [see Fig. 3(a)], which suggests that the structural changes at lead head precede those at rear head. So we infer that Pi release at lead head is favored over ADP release at rear head, which leads to (A-M-ADP, A-M-ADP) state. Therefore, despite the hindrance of backward strain, the lead head rapidly binds with F-actin and releases Pi, while the rear head remains ADP-bound. To further explore how much the strain affects the transitions at rear and lead head, we redo transition pathway modeling from (A-M-ADP, A-M-ADP-Pi) state to (A-M, A-M-ADP) state without imposing the distance constraint that causes strain. Then we compare the reaction coordinates of rear and lead head in the presence and absence of strain [see Fig. 3(a)]. We find that the strain lowers the reaction coordinate of lead head along the entire transition pathway but increases the reaction coordinate of rear head during the early part of the transition pathway [see Fig. 3(a)]. Therefore, the strain delays the transition at lead head (Pi release) more than it advances the transition at rear head (ADP release) [see Fig. 3(a)]. However, the strain effect is not enough to change the order of fast Pi release versus slow ADP release intrinsic to monomeric myosin V.<sup>19</sup>

Next, we start from (A-M-ADP, A-M-ADP) state and determine whether ADP release occurs next at lead head or rear head. Our analysis of the transition pathway from (A-M-ADP, A-M-ADP) state to (A-M, A-M) state indicates that the structural changes at rear head precede those at lead head [see Fig. 3(b)]. So we infer that ADP release at rear head is favored over lead head, which leads to (A-M, A-M-ADP) state. The differentiation in ADP release at two myosin heads stems from the action of forward/backward pulling force on rear/lead head. To further explore how much the strain affects the transitions at rear and lead head, we redo transition pathway modeling from (A-M-ADP, A-M-ADP) state to (A-M, A-M)

state without imposing the distance constraint that causes strain. Then we compare the reaction coordinates of rear and lead head in the presence and absence of strain [see Fig. 3(b)]. We find that the strain significantly delays the transition at lead head but only slightly advances the transition at rear head [see Fig. 3(b)]. This finding agrees with mechanical studies of monomeric myosin V, which found that pulling forward produces only a small change in kinetics (i.e., ADP dissociation is modestly accelerated<sup>18</sup>), whereas pulling backward induces a large change in kinetics (i.e., ADP dissociation is inhibited<sup>23,110</sup>).

Taken together, we have derived the following favorable sequence of transitions associated with the release of hydrolysis products in myosin V dimer: (A-M-ADP, A-M-ADP-Pi)  $\rightarrow$  (A-M-ADP, A-M-ADP)  $\rightarrow$  (A-M, A-M-ADP). Namely, Pi is rapidly released at lead head followed by ADP release at rear head, while ADP release at lead head is inhibited by backward strain. Our finding supports the kinetic coordination model based on fast Pi release at lead head, slow ADP release at rear head, and inhibited ADP release at lead head.<sup>21–24</sup>

# Decrypting sequence of structural changes during transitions of myosin V dimer

Finally, we will use the iENM protocol<sup>84</sup> to explore the detailed sequence of structural changes in a myosin V dimer during three key transitions that accompany Pi release, ADP release, and ATP binding. Our goal is to dissect the strain dependence of the allosteric couplings among nucleotide-binding site, actin-binding cleft, and converter at different stages of kinetic cycle of myosin V.

To probe the transition that accompanies Pi release at lead head, we model the transition pathway from (A-M-ADP, A-M-ADP-Pi) state to (A-M-ADP, A-M-ADP) state. Then we analyze the reaction coordinates associated with three key parts of lead head: actin-binding cleft (represented by residues 492-533 of HLH motif, residues 376-378, and 390-392 of CM loop; see Fig. 1), nucleotidebinding site (represented by residues 214-219 of switch I, residues 442-448 of switch II, see Fig. 1), L50 subdomain (residues 449-565) and converter (residues 698-752). We find a sequence of structural changes led by the closing of actin-binding cleft, followed by the rotation of converter (relative to L50), and the relative motion between switch I and II [see Fig. 3(c)]. The above order is consistent with the causal relation between actin binding and Pi release (via a backdoor between switch I and II; Refs. 86,106). To further explore how much the strain affects the transition at lead head, we redo transition pathway modeling from (A-M-ADP, A-M-ADP-Pi) state to (A-M-ADP, A-M-ADP) state without imposing the distance constraint that causes strain. Then we compare the reaction coordinates in the presence and absence of backward strain [see Fig. 3(c)]. We find that the backward strain significantly delays the motions of converter

and switch I-II but has less effect on the closing of actinbinding cleft [see Fig. 3(c)]. So we infer that the lead head can still rapidly form strong actin-myosin bond although its converter is rotated backward by strain. Therefore, the strong coupling between closing of actinbinding cleft and rotation of converter, as found for monomeric myosin motor domain,<sup>86</sup> seems to be weakened in the presence of backward strain. Our finding that the strain delays switch I-II motion may explain the finding of  $\sim 100$  times slower Pi release at the lead head when the rear head is bound with ADP (with more strain) than when it is nucleotide-free (with less strain).<sup>25</sup> However, another kinetic study found Pi release to be fast regardless of strain<sup>21</sup>. It is possible that Pi may be released via alternative paths instead of the backdoor.<sup>50</sup> Further studies are needed to resolve this issue.

To probe the transition that accompanies ADP release at rear head, we model the transition pathway from (A-M-ADP, A-M-ADP) state to (A-M, A-M-ADP) state. Then we analyze the reaction coordinates associated with three parts of rear head: actin-binding cleft, nucleotidebinding site (represented by residues 214-219 of switch I, residues 163-170 of P loop; see Fig. 1), L50 subdomain and converter. We find a sequence of structural changes beginning with the downward rotation of converter, followed by further closing of actin-binding cleft, and finally the opening of switch I (relative to P loop) that allows ADP release [see Fig. 3(d)]. The above sequence is compatible with the causal relation between actin binding and ADP release. To further explore how much the strain affects the transition at rear head, we redo transition pathway modeling from (A-M-ADP, A-M-ADP) state to (A-M, A-M-ADP) state without imposing the distance constraint that causes strain. Then we compare the reaction coordinates in the presence and absence of forward strain [see Fig. 3(d)]. We find that the forward strain significantly advances the rotation of converter but has little effect on the closing of actin-binding cleft and the opening of switch I relative to P loop [see Fig. 3(d)]. So we infer that ADP release at rear head is triggered by further closing of the actin-binding cleft, and both are weakly coupled to the downward rotation of converter. This is in agreement with mechanical studies that found small effect of forward pulling on kinetics.<sup>23,110</sup> Our finding suggests that the reciprocal coupling between actin bind-ing and nucleotide unbinding $^{87-90}$  is involved in both Pi release and ADP release.

To probe the transition that accompanies ATP binding at rear head, we model the transition pathway from (**A-M**, **A-M-ADP**) state to (**A-M-ATP**, **A-M-ADP**) state. Then we analyze the reaction coordinates associated with three parts of rear head: actin-binding cleft, nucleotidebinding site (represented by residues 214–219 of switch I, residues 163–170 of P loop; see Fig. 1), L50 subdomain and converter. We find a sequence of structural changes starting with the closing of switch I (relative to P loop),

followed by the rotation of converter, and finally the opening of actin-binding cleft [see Fig. 3(e)]. The above sequence reveals how ATP binding induces a chain of structural events that reposition converter and cause the dissociation of myosin from F-actin. Our prediction qualitatively agrees with a recent NMA modeling,<sup>83</sup> which predicts that ATP binding triggers a change in switch I-P loop interaction, which then propagates to Nterminal subdomain, converter and L50 subdomain, and finally leads to the opening of actin-binding cleft. To further explore how much the strain affects the transition at rear head, we redo transition pathway modeling from (A-M, A-M-ADP) state to (A-M-ATP, A-M-ADP) state without imposing the distance constraint that causes strain. Then we compare the reaction coordinates in the presence and absence of forward strain [see Fig. 3(3)]. We find that the forward strain has little effect on the movements of all three parts [see Fig. 3(e)]. So we infer that ATP binding induces the detachment of rear head from F-actin, which is not affected by strain.

In sum, we have predicted a detailed sequence of structural changes during Pi release at lead head, ADP release, and ATP binding at rear head. Our modeling has elucidated the allosteric coupling between actin binding and nucleotide binding in myosin at different stages of kinetic cycle: the allostery signal is transmitted from the actin-binding cleft to the nucleotide-binding site during Pi release and ADP release, giving rise to actin-activated release of hydrolysis products; the direction of signaling is reversed during ATP binding, allowing ATP-activated dissociation of myosin from F-actin. The above predictions may be tested by the  $\Phi$ -value analysis of kinetics of mutant proteins as done for ligand-gated ion channels<sup>120</sup>—residues with high/low  $\Phi$  values undergo early/ late structural changes during a transition.

Before ending, we further discuss the following additional issues.

#### Multiple ADP states

There is kinetic<sup>121,122</sup> and mechanical<sup>23</sup> evidence for the existence of at least two actomyosin ADP states which differ in actin-binding and ADP-binding affinity. Here we only model the first ADP state (with moderate affinity for actin and strong affinity for ADP) while the second one (with strong affinity for actin and weak affinity for ADP) is combined with the A-M state due to their structural similarity.<sup>35</sup> A third ADP state of myosin V with the actin-binding cleft partially closed and the lever arm in the pre-powerstroke position was proposed based on kinetic studies<sup>21,24</sup> and theoretical modeling.<sup>102,123</sup> Such ADP state was needed to interpret the EM finding of two-head-bound myosin V conformations with the lead-head lever arm in the pre-powerstroke position.<sup>100,101</sup> As shown by our modeling, the pre-powerstroke conformation of lever arm can be induced by applying backward strain to the **A-M-ADP** state model which adopts an intermediate lever-arm orientation [see Fig. 2a,b)]. So it is not necessary to introduce an additional pre-powerstroke ADP state in our modeling.

## Stiff lever arm

The long lever arm of myosin V acts as a lever to generate a work stroke proportional to its length.<sup>109,124,125</sup> So it is reasonable to think that a stiff lever arm (with high persistent length) is required to fulfill its mechanical role. Indeed, high stiffness was obtained for myosin V lever arm by several previous studies (although low stiffness was also explored; see Refs. 103,126). In a mechanochemical modeling of myosin V, a persistent length  $\sim$ 375 nm was obtained based on the fitting of step size of myosin V under load.<sup>102</sup> A mechanochemical model was used to fit measured relaxation distance and stall force of myosin V, which also obtained a high persistent length  $\sim 500$ nm (see Ref. 127). The fitting of step size versus number of IQ motifs obtained a persistent length of  $\sim$ 310 nm.<sup>124</sup> In this study, we fit the persistent length of 375 nm by choosing  $R_c = 40$  Å for pairs of residues within the lever arm and converter, which significantly stiffens these mechanical parts compared with the rest of motor domain. A stiff lever arm has the following important implications:

- a. It favors straight lever arm over strongly curved lever arm, which is in agreement with recent EM,<sup>100,101</sup> AFM,<sup>95</sup> and fluorescence imaging studies.<sup>115,119</sup>
- b. It is required for the strain-induced differentiation of ADP release at lead and rear head. Indeed, if we reduce lever-arm stiffness by lowering  $R_c$  to 20 Å, the difference in reaction coordinates of lead and rear head during ADP release becomes much smaller (see Supporting Information Fig. S2), suggesting weakened differentiation between ADP release at rear and lead head. A recent free energy analysis of myosin V also found the high stiffness of lever arm is important for interhead coordination.<sup>123</sup>

It was shown previously that function of myosin V is compromised after the dissociation of light chains that reduces the lever-arm stiffness.<sup>128</sup> To further test the importance of stiff lever arm in kinetic coordination, one can introduce point mutations (such as mutating to proline) to lever arm that reduce its stiffness and then check if the processive stepping of myosin V dimer is compromised.

#### Relation to other modeling studies of myosin V

Our study complements previous theoretical studies of myosin V stepping by discrete stochastic models,<sup>26,129–131</sup> mechanochemical models,<sup>102,123,126,127</sup> and other coarse-grained modeling,<sup>132</sup> which offer great insights to the kinetics of myosin V but lack fine details of structural

changes and full consideration of protein flexibility. Our coarse-grained modeling not only provides unprecedented structural details at residue level but also allows modeling of elasticity and flexibility of entire myosin V (and part of F-actin). Our residue-level models of monomeric and dimeric actomyosin V can be further refined to atomic resolution, which will open door for atomistic simulations that may probe the intricacy of dynamic interactions and fluctuations within myosin V.

# Actomyosin kinetics is limited by conformational transitions

Our modeling is based on the assumption that the kinetic steps of actomyosin V are limited by the local and global conformational changes in myosin rather than ligand diffusion. Indeed, the rate of nucleotide diffusion is a few microns per millisecond,<sup>133</sup> so the rate of a nucleotide diffusing in or out of the active site is of the order of  $10^6 \text{ s}^{-1}$ . In contrast, the rates of various kinetic steps of actomyosin V are much lower—ranging from 10 to  $10^3 \text{ s}^{-1}$ .<sup>19</sup>

Before ending, we highlight the following experimentally testable results from our modeling:

- a. The highly processive stepping of myosin V dimer depends on a stiff lever arm. Therefore, by introducing point mutations to the lever arm to fine tune its stiffness, one can modify the processivity of myosin V.
- b. The structural motions of various parts of myosin V follow specific orders during the conformational transitions. Such motional orders can be probed by the  $\Phi$ -value analysis of kinetics of mutant proteins<sup>120</sup> or using florescence probes.<sup>134</sup>

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