Multiscale molecular dynamics simulations of rotary motor proteins

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Received: 4 October 2017 / Accepted: 23 November 2017 / Published online: 4 December 2017

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Abstract

Protein functions require specific structures frequently coupled with conformational changes. The scale of the structural dynamics of proteins spans from the atomic to the molecular level. Theoretically, all-atom molecular dynamics (MD) simulation is a powerful tool to investigate protein dynamics because the MD simulation is capable of capturing conformational changes obeying the intrinsically structural features. However, to study long-timescale dynamics, efficient sampling techniques and coarse-grained (CG) approaches coupled with all-atom MD simulations, termed multiscale MD simulations, are required to overcome the timescale limitation in all-atom MD simulations. Here, we review two examples of rotary motor proteins examined using free energy landscape (FEL) analysis and CG-MD simulations. In the FEL analysis, FEL is calculated as a function of reaction coordinates, and the long-timescale dynamics corresponding to conformational changes is described as transitions on the FEL surface. Another approach is the utilization of the CG model, in which the CG parameters are tuned using the fluctuation matching methodology with all-atom MD simulations. The long-timespan dynamics is then elucidated straightforwardly by using CG-MD simulations.

Keywords Molecular dynamics simulation · Free energy calculation · Coarse-grained molecular dynamics simulation · F_1 -ATPase · V_1 -ATPase

Multiscale approach for analyzing conformational change in proteins

To understand protein functions, it is necessary to apply a bridging approach between atomic dynamics and domain or subunit-level motions. For example, in rotary ATPases, the rotation of the central stalk was observed by single-molecule experiments, with the order of rotation dynamics determined to be approximately sub-milliseconds (Okuno et al. 2011; Iino et al. 2015). In the motor ATPases, nucleotide events, i.e., ATP (adenosine triphosphate) binding, hydrolysis, and adenosine diphosphate inorganic phosphate ion (ADP/Pi) release, occur at the atomic level, and the atomic-level events cause a series of large-scale conformational changes in the subunits. To bridge the gap between the atomic motions and large-scale

dynamics, it would be useful to apply physicochemical approaches, such as molecular dynamics (MD) simulation.

With steadily increasing computational power becoming available, MD simulation is becoming a powerful tool to study protein dynamics (Dror et al. 2012; Lane et al. 2013; Goh et al. 2016). However, conventional all-atom MD simulation suffers from two major obstacles. The first is the limitation of the timescale, and the second is the accuracy of the force fields. To overcome the limitation of the timescale, much effort has been expended to improve MD calculations by, for example, developing highly parallelized algorithms [e.g., GROMACS (Abraham et al. 2015), AMBER (Case et al. 2017), NAMD (Phillips et al. 2005), and GENESIS (Kobayashi et al. 2017)] and specialized hardware [e.g., MD-GRAPE (Ohmura et al. 2014) and ANTON (Shaw et al. 2008)]. In addition to conventional MD simulations, efficient sampling techniques have been developed with biased simulations [e.g., replica exchange (Sugita and Okamoto 1999), accelerated MD (Hamelberg et al. 2004), string methods (Weinan and Vanden-Eijnden 2010), and metadynamics (Piana and Laio 2007)] and statistical analysis of unbiased simulations [e.g., the Markov state model (Chodera and Noe 2014) and weighted ensemble simulation

This article is part of a Special Issue on ‘Biomolecules to Bio-nanomachines—Fumio Arisaka 70th Birthday’ edited by Damien Hall, Junichi Takagi and Haruki Nakamura

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(Zuckerman and Chong 2017)]. The accuracy of the force fields is another challenge to be met in MD simulations. Comparison of the simulated dynamics with those observed in experiments allows us to assess the accuracy of the force fields (see Beauchamp et al. 2012; Lindorff-Larsen et al. 2012; Rauscher et al. 2015), and the improvements of force fields are still ongoing. Although the all-atom treatment describes protein dynamics at the atomic level, sufficient sampling is often expensive in terms of computation costs. Therefore, coarse-grained (CG) models (Saunders and Voth 2013) are utilized to reduce the computational burden. For example, the CG-MD software CafeMol (Kenzaki et al. 2011) employs a CG model in which one amino acid residue is represented by one bead located on its C α atom, and the solvent is treated as a continuum dielectric model. Owing to the drastic decrease in the number of simulated particles, CG-MD simulations can be performed in substantially fast computations.

Multiscale MD simulation is a promising approach for gaining an understanding of the proper mechanism underlying the large-scale dynamics in terms of the atomic picture. This review focuses on two approaches. The first is free energy landscape (FEL) analysis (Ito et al. 2011; Ito and Ikeguchi 2015), and the second is CG-MD simulation tuned by the methodology of fluctuation matching with all-atom MD simulations (Isaka et al. 2017). In FEL analysis, the potential mean force (PMF) on a reaction coordinate, i.e., FEL along the reaction coordinate, is obtained using a path generation technique and a sampling technique with biased all-atom MD simulations. Large-scale conformational changes coupled with atomic motions can be understood as transitions on the FEL surface. Although FEL analysis provides an atomic-resolution picture, the computational cost is still high, and a theoretical approach is also employed to obtain the thermodynamic picture (Yoshidome et al. 2011, 2012). In the second approach, CG-MD simulations are coupled with all-atom MD simulations. In the fluctuation matching methodology, the structural fluctuations around a minimum in the CG potential are optimized to match fluctuations observed in all-atom MD simulations. According to the picture presented by the linear response theory (Ikeguchi et al. 2005), protein fluctuations are crucial for the conformational changes induced by ligand binding because the structural change upon ligand binding is achieved as a response behavior related to equilibrium fluctuations of the ligand-free state. This implies that the structural and dynamical features in proteins determine their own molecular functions.

Here, we review two examples of MD simulations, both of which are rotary ATPases (Stewart et al. 2014): one is ATP synthase F-ATPase expressed in mitochondria; the other is vacuolar ATPase (V-ATPase) expressed in organelles and membrane vesicles, which regulates the acidic environment coupled with ATP hydrolysis. Both ATPases have similar overall structures, consisting of two motor and stator subunits (Fig. 1a). The motors are a proton (or ion) pump moiety F_O (or

V_O for V-ATPase; hereafter, the corresponding variable for V-ATPase is written in parenthesis) embedded in membranes and a soluble moiety F_1 (or V_1) in which a central stalk penetrates the center of the asymmetry-packed $\alpha_3\beta_3$ (or A_3B_3) subunits, in which three catalytic β (or A) subunits and three non-catalytic α (or B) subunits are arranged alternatively (Figs. 1a, 2a). Using the gradient of the electrostatic potential and proton concentration, F_O rotates and drives F_1 to synthesize ATP from ADP and P_i through the rotation of the central stalk. The reverse reaction is possible in F-ATPase; i.e., ATP hydrolysis drives the rotation of the central stalk, leading to the active transport of protons. In V-ATPase, the physiological function is the active transport of protons or ions driven by ATP hydrolysis. The isolated soluble parts of F-ATPase and V-ATPase, i.e., F_1 -ATPase and V_1 -ATPase, exhibit the stalk rotation induced by ATP hydrolysis (Okuno et al. 2011). This review focuses on the rotation behaviors of F_1 -ATPase and V_1 -ATPase driven by ATP hydrolysis.

The stalk rotation through ATP hydrolysis has been studied by single-molecule experiments (Okuno et al. 2011; Iino et al. 2015). A 360° rotation occurs in approximately milliseconds coupled with three ATP hydrolysis events. Three main pause steps consisting of 120° intervals were observed during the rotation. In bacterial F_1 -ATPase, the 120° rotation in association with ATP hydrolysis is achieved by substeps of 80° and 40°, corresponding to ATP binding and ATP hydrolysis, respectively. By contrast, in V_1 -ATPase, no substeps in the 120° rotation were observed. This result implies that the rotation mechanisms in the two ATPases differ somewhat from each other (Imamura et al. 2005).

An important question is how the chemical events, i.e., ATP binding, hydrolysis, and product release, lead to the mechanical event, i.e., the stalk rotation. Each chemical event induces a conformational change of the catalytic subunit. Upon ATP binding, the catalytic subunit undergoes a closing motion. Then, after ATP hydrolysis, an opening motion of the subunit occurs (Masaike et al. 2008). Product release also induces a conformational change of the subunit. These conformational changes in the subunit influence adjacent subunits in the $\alpha_3\beta_3$ (or A_3B_3) subunits. Consequently, the cooperative motions of the subunits induce stalk rotation. Therefore, to elucidate the rotation mechanism in terms of their structures, it is important to investigate the multiscale connections from detailed atomic interactions between nucleotide and protein to large-scale cooperative motions of subunits.

Investigation of conformational changes for F_1 -ATPase with FEL analyses and thermodynamic analyses

Based on integrated insights from crystallography and single-molecule experiments (Yasuda et al. 1998, 2010;

Shimabukuro et al. 2003; Kabaleeswaran et al. 2006; Adachi et al. 2007; Masaike et al. 2008; Okuno et al. 2008, 2011; Watanabe et al. 2010; Watanabe and Noji 2013;), a general picture of the rotation mechanism of F_1 -ATPase coupling with ATP hydrolysis has been elucidated, as follows (Fig. 1a). In the ATP-binding dwell state, three catalytic $\alpha\beta$ pairs separately adopt the open (E) structure, the half-closed (HC) structure, and the closed (TP) structure. Here, no nucleotide is bound to the E structure, ADP and Pi are bound to the HC structure, and ATP is bound to the TP structure. Upon ATP binding to the E structure, ADP dissociation occurs at the HC structure. The ATP binding induces a closing motion from the E structure to the TP structure, and the ADP dissociation induces an opening motion from the HC structure to the open (E·Pi) structure. Additionally, these conformational changes induce a closing motion from the TP structure to the tightly closed (DP) structure. These correlated motions of the $\alpha\beta$ subunits lead to the 80° rotation of the stalk, and the state shifts to the catalytic dwell state. The ATP hydrolysis at the DP structure induces an opening motion from the DP structure to the HC structure. The Pi release loosens the interface of the open structure, leading to further rotation of the stalk, as suggested by the crystal structures of yeast F_1 -ATPase (Kabaleeswaran et al. 2006). Then, the state shifts back to the ATP-binding dwell, whose structure is a 120° rotated structure of the starting point. Focusing on the catalytic β subunit, during 120° rotation, the subunit undergoes a closing motion through the E, TP, and DP structures and an opening motion through the HC, E·Pi, and E structures. Although these general scenarios were obtained from snapshot structures in the different states as revealed by crystallography, as described above, the detailed mechanism of the conformational changes induced by atomic interactions between nucleotide and protein remains elusive.

Detailed mechanisms for the conformational change of the subunits have been revealed at atomic resolution using FEL analysis with all-atom MD simulations (Ito et al. 2011; Ito and Ikeguchi 2015). FEL calculation was carried out by the combined use (Arora and Brooks 2007, 2009) of the nudged elastic band (NEB) method (Jonsson et al. 1998; Chu et al. 2003), to create an initial path of the conformational changes, and the umbrella sampling method (Torrie and Valleau 1974), to calculate the PMF along the reaction coordinate. Following the generation of intermediate structures between the given end-point structures by a linear interpolation, the NEB method provided an energy-minimum path between them via the adopted basis Newton–Raphson minimization. Then, a reaction coordinate was determined to characterize the conformational change. In this study, the difference in the root-mean-square deviation (RMSD) from the two end-point structures ΔD_{rmsd} was employed for the reaction coordinate. Umbrella sampling was carried out using intermediate structures at various ΔD_{rmsd} along the path. From each intermediate structure, all-atom MD simulation was conducted with a restraint of the

harmonic potential so that the structural ensemble around the given ΔD_{rmsd} was sampled. From a series of MD simulations for various ΔD_{rmsd} , a PMF along the reaction coordinate, i.e., the FEL, was calculated by the weighted histogram analysis method (Kumar et al. 1992). The obtained one-dimensional (1-D) FEL provided information about not only the stable structure at the global minimum but also on the stable intermediate structures along the path. Additionally, the PMF could be mapped on a 2-D surface of the other structural feature and ΔD_{rmsd} , allowing us to examine how the structural feature is related to the conformational changes in terms of FEL.

To understand the detailed mechanism of the closing motion from the open (E) to the closed (TP) structures induced by ATP binding, Ito et al. (2011) used the isolated catalytic β subunit system to obtain the FELs between the two structures in both the ATP-free state and the ATP-bound state (Fig. 1b, c). ΔD_{rmsd} is defined as the difference in two RMSDs from the closed and open structures, and a negative ΔD_{rmsd} indicates that the structures are close to the open structure. In the FEL for the ATP-free state (Fig. 1b), the open structure is more stable than the closed structure. By contrast, in the FEL for the ATP-bound state (Fig. 1c), the closed structure is stable. This result shows that the closing motion occurs when ATP is bound and that the ATP binding to the open structure is a trigger of the closing motion. The stability of the open structure for the ATP-free state was also examined using all-atom MD simulations for the isolated β subunit. The β subunit kept an open structure when no nucleotide was bound (Ito and Ikeguchi 2010a). Once an ATP binds to the open structure, the closing motion occurs through several intermediate structures. The free-energy surface for the ATP-bound state exhibits three minima (denoted as i, ii, and iii in Fig. 1b) and a barrier at $\Delta D_{\text{rmsd}} \sim 0$. From the 2-D free-energy surfaces, structural features related to the conformational changes along the energy surface were indicated, as shown in Fig. 1d (Ito et al. 2011). From minimum i to minimum ii, the hydrogen bond network around ATP was changed, and the change induced structural stresses. In particular, the hinge region, β -sheets 3 and 7, and the B-helix, are forced to change their conformations in the uphill region from minimum ii to the barrier. The stressed structure relaxed in the downhill region from the barrier to the global minimum iii through the sliding motion of the B-helix, resulting in the closing motion of the C-terminal region.

The detailed mechanism of the opening motion from the closed (DP) structure to the open (E·Pi) structure after ATP hydrolysis was investigated by FEL analysis (Ito and Ikeguchi 2015). Both ADP- and Pi-bound DP structures were obtained just after ATP hydrolysis using the hybrid quantum mechanics/molecular mechanics (QM/MM) simulation (Hayashi et al. 2012), in which chemical reactions for ATP were incorporated. The FELs between the DP structure and

the open structure for the $\alpha\beta$ subunits were then calculated in both the ADP- and Pi-bound (ADP + Pi-bound) state and only the ADP-bound state (Fig. 1e, f) (Ito and Ikeguchi 2015). The FEL for the ADP + Pi-bound state showed a single minimum (Fig. 1e). Comparison of the structure at the global minimum with the HC structure in the crystal structure based on the

RMSD and the orientation of helix 6 revealed that the minimum structure was in good agreement with the HC structure, indicating that the HC structure spontaneously emerges in the ADP + Pi-bound state (Fig. 1e). By contrast, when Pi was dissociated, the opening motion was inhibited due to the large barrier between the closed and open structures (Fig. 1f),

Fig. 1 Rotation behavior of F_1 -ATPase and main results of molecular dynamics (MD) simulations. **a** Reaction scheme of the 120° rotation. Intermediate structures are in black-colored brackets. General information of F_1 -ATPase is in the rectangular inset (Ito et al. 2013). **b** The free energy landscape (FEL) of the ATP-bound state (Ito et al. 2011). **c** The FEL of the ATP-free state (Ito et al. 2011). **d** Summary of the structural mechanism in the closing motion of the β subunit (Ito et al. 2011). **e** The FEL of the ADP- and inorganic phosphate (Pi)-bound state (Ito and Ikeguchi 2015). **f** The FEL of the ADP-bound state (Ito and Ikeguchi 2015). **g** Summary of the structural mechanism in the opening motion of the $\alpha\beta$ pair (Ito and Ikeguchi 2015). **h** Summary of the stable contacts. Red- and gray-colored lines represent collective motions of C-terminal and nucleotide-binding domains, respectively (Ito and Ikeguchi 2010a, b). **i** Schematic picture of the water-entropy gain owing to the decrease of the excluded volume of the protein (Yoshidome et al. 2011). **j** Summary of rearrangement dynamics upon Pi release (Ito et al. 2013). **a, j** Reprinted with permission from Ito et al. (2013), copyright 2013 American Chemical Society. **b, c, d** Reprinted with permission from Ito et al. (2011), copyright 2011 American Chemical Society. **i** Reprinted with permission from Yoshidome et al. (2011), copyright 2011 American Chemical Society. See text for details

suggesting that the subunit remains in the closed structure. This result is consistent with single-molecule experiments (Watanabe et al. 2010) and theoretical studies (Okazaki and Hummer 2013) suggesting that Pi remains at the nucleotide-binding site and dissociates at the next step. The FEL of the ADP + Pi-bound state (Fig. 1e) shows that the opening motion is achieved by two steps corresponding to the downhill/uphill regions in the FEL (Fig. 1g). In the downhill region from the closed structure to the HC structure, B-helix sliding occurred via a separation of ADP and Pi; ADP bound to the P-loop near the B-helix, whereas Pi bound to the upper helix, and then the separation of ADP and Pi influenced the position of the B-helix sliding. In the uphill region from the HC structure to the open structure, ADP dissociation occurred due to the conformational change of the P-loop induced by the change in the hydrogen bond of Lys162. This motion in the uphill region should be coupled with the closing motion of the adjacent catalytic pair because such a closing motion from the open to the TP structures corresponds to the downhill region of the energy surface.

In addition to detailed studies on the mechanism of the closing/opening motions, the rotation mechanism of whole F_1 -ATPase were studied using a combination of water-entropy analysis based on statistical thermodynamic theory and structural fluctuation analysis by all-atom MD simulations (Ito and Ikeguchi 2010a, b; Yoshidome et al. 2011). Reflecting the asymmetrically packed structure, stable contacts between adjacent subunits were observed, especially in tightly packed subunits around the DP structure, and few stable contacts were observed in loosely packed subunits around the open (E) structure (Fig. 1h). These different packing features gave rise to the different structural fluctuations: The tightly packed regions exhibited suppressed fluctuation, and the loosely packed regions exhibited significant fluctuation. The packing feature

of proteins is mainly realized by hydrophobic effects, i.e., the water-entropy gain (Fig. 1i). Thus, the water entropy was theoretically estimated by the morphometric approach (Roth et al. 2006), in which a thermodynamic quantity was expressed by a linear combination of four terms depending on the structural features of the solute (e.g., the excluded volume and the solvent accessible surface area). Using the crystal structure of F_1 -ATPase, the water-entropy gain corresponding to the water-entropy change upon the formation of the pair structure was estimated, and the water-entropy gain was highly correlated with the tightness of the pair characterized by stable contacts. Taken together, the rotation mechanism can be understood as follows: when ATP is hydrolyzed, the asymmetrically packed $\alpha_3\beta_3$ complex loosens the interfaces at the ATP hydrolysis site, thereby reducing the water entropy. To compensate for the loss of entropy, the stalk rotates, and the other interfaces are tightened, which yields water-entropy gain. Finally, the $\alpha_3\beta_3$ complex readopts the asymmetric packing, consisting of tightly packed (DP), moderately packed (TP), and loosely packed (E) pairs because the structure is the most stable in terms of water entropy. This mechanism is referred to as the packing exchange mechanism (Ito and Ikeguchi 2014).

The packing exchange mechanism in the 16° rotation observed in the crystal structures of yeast F_1 -ATPase has been examined (Yoshidome et al. 2012). To elucidate structural fluctuations, MD simulations were conducted and showed that the DP structure adopted the tightest packing in the catalytic dwell state; by contrast, in the structure after the 16° rotation of the stalk, the TP structure adopted the tightest packing (Ito et al. 2013). This difference was caused by the different positions of the stalk and the loosening of the interface of the open structure due to the Pi release, which indirectly loosens the DP interface and tightens the TP interface (Fig. 1j).

In summary, a series of studies (Ito et al. 2011; Yoshidome et al. 2011, 2012; Ito and Ikeguchi 2015) has revealed the detailed structural mechanism of the closing/opening motions of the catalytic subunit (pair) at atomic resolution. Using free-energy simulations, several crucial events were detected along the transition path. From a combination analysis of structural fluctuation and water entropy, the rotation mechanism, in terms of the packing exchange of the asymmetrically packed $\alpha_3\beta_3$ complex, was provided. Following ATP hydrolysis, the catalytic subunit pair undergoes conformational changes, thereby loosening the interface, leading to water-entropy loss. To compensate for the loss, stalk rotation and cooperative rearrangements of subunit pairs occur so that the asymmetrically packed structure is recovered because the tightly packed interfaces yield water-entropy gain. In this scenario, a crucial role is played by the asymmetrically packing structure of the $\alpha_3\beta_3$ complex, rather than by specific interactions between the stalk and the $\alpha_3\beta_3$ complex. This mechanism is supported by experimental results (Furuike et al. 2008), suggesting that

the stalk rotation occurs even when most of the penetrated part of the stalk is removed, thereby implying that the stalk rotation can be induced mainly by conformational changes of the $\alpha_3\beta_3$ complex.

Investigation of the stalk rotation for V_1 -ATPase by multiscale MD simulations

In contrast to the accumulated structural information on F_1 -ATPase, a high-resolution structure of V_1 -ATPase was determined only very recently. In 2013 and 2016, asymmetric crystal structures of *Enterococcus hirae* V_1 -ATPase were published (Arai et al. 2013; Suzuki et al. 2016), and taken together with single-molecule experiments (Iino et al. 2015), a possible model of the 120° stalk rotation was proposed. Three important crystal structures of V_1 -ATPase were resolved: the catalytic dwell ($2_{ATP}V_1$), the ATP-binding dwell ($2_{ADP}V_1$), and the ADP-release dwell ($3_{ADP}V_1$). In this model, coupled with ATP hydrolysis, the A_3B_3 complex undergoes a series of conformational changes, with states $2_{ATP}V_1$, $2_{ADP}V_1$, $3_{ADP}V_1$, and $2_{ATP}V_1$ occurring in turn upon the stalk rotation (Fig. 2a). The catalytic subunit of V_1 -ATPase is the A subunit, whereas that of F_1 -ATPase is the β subunit. For non-catalytic subunits, the B subunit of V_1 -ATPase corresponds to the α subunit of F_1 -ATPase. Here, the last $2_{ATP}V_1$ is a 120°-rotated structure of the initial $2_{ATP}V_1$ and, hereafter, the subunits are referred to as A_I , B_I , etc., as shown in Fig. 2a. Before ATP hydrolysis, three AB subunit pairs adopt the open, moderately closed, and tightly closed structures, termed as the empty, bound, and tight structures, respectively. When ATP hydrolysis and Pi release occur at the $A_{III}B_{III}$ pair, the tight structure of the $A_{III}B_{III}$ pair is loosened. Additionally, the $A_I B_I$ pair undergoes a conformational change to the “bindable-like” structure that has a high affinity for ATP. Following ATP binding to the bindable-like structure, the $A_I B_I$ pair undergoes a closing motion. In contrast, ADP dissociation from the $A_{III}B_{III}$ pair induces an opening motion. These correlated motions of A_3B_3 subunits may induce the stalk rotation.

To understand the rotation mechanism of V_1 -ATPase, multiscale MD simulations were performed for analysis of cooperative motions among the three AB pairs and the stalk (Isaka et al. 2017). Due to the sub-millisecond order of the stalk rotation (Iino et al. 2015), conventional all-atom MD simulations are beyond the timescale. Therefore, CG-MD simulations in combination with all-atom MD simulations using the fluctuation matching methodology were employed to overcome the limit of the timescale. To optimize the parameters of the CG-MD simulations, the fluctuation matching methodology was employed in this study so that the fluctuations of CG residues around a minimum were matched to those of all-atom MD simulations at equilibrium near the crystal structure. Then, the stalk rotation was examined using a

multiple-Go model (Okazaki et al. 2006; Okazaki and Takada 2008; Yao et al. 2010; Kenzaki et al. 2011), in which two minima were set at the structures before and after ATP hydrolysis, corresponding to the $2_{ATP}V_1$ crystal structure and its 120°-rotated structure, respectively.

The CG parameters were tuned using only the CG temperature (T_{CG}). Several CG-MD simulations with various T_{CG} were performed; the root-mean-square fluctuations (RMSFs) at each T_{CG} were compared with those of all-atom MD simulations of the $2_{ATP}V_1$ crystal structure at 300 K, and it was determined that at $T_{CG} = 240$ K, the RMSF of CG-MD was in good agreement with those of the all-atom MD simulation (Fig. 2b), indicating that the fluctuations around the minimum in the CG-MD simulations are close to those in the all-atom MD simulations. This agreement is not surprising because the employed CG potential [the atomic-interactionbased coarse grained (AICG model)] is designed on the basis of atomic interactions (Li et al. 2011).

Using the determined T_{CG} and the multiple-Go model, several CG-MD simulations have provided a successful stalk rotation. The time evolution of the stalk rotation in a CG-MD simulation is illustrated in Fig. 2d. As the simulation proceeded, the stalk showed a 120° rotation with no substep, which is consistent with the rotation behavior observed in experiments. Because single-molecule experiments showed that a 120° rotation is completed within 0.2 ms (Iino et al. 2015), $\sim 100 \times 10^3$ steps for a 120° rotation in the CG-MD simulations may correspond to approximately sub-milliseconds. To examine the conformation of the AB subunit during the rotation, RMSDs from several structures observed in the crystal structure were calculated (Fig. 2c). The $A_I B_I$ subunit adopted the empty structure before rotation and changed to the bound structure after rotation. Surprisingly, the $A_I B_I$ subunit adopted the bindable-like structure just before the beginning of the stalk rotation. The minimum RMSD from the bindable-like structure was 1.36 Å, suggesting that the structure emerging during the simulations is very close to the bindable-like structure in the crystal structure. The CG-MD simulations were repeatedly performed, and the bindable-like structure was observed in all of the CG-MD simulations. Note that this emergence is not trivial because the bindable-like structure was not used as the input structure.

The spontaneous emergence of the bindable-like structure was induced by the cooperative rearrangements of the B_I and A_{III} subunits (Fig. 2d, panel B). First, the $A_{III}B_{III}$ pair underwent conformational changes, and the A_{III} subunit moved outward. Due to the interaction between the A_{III} and B_I subunits, the A_{III} subunit pulled the B_I subunit. The outside movement of the $A_{III}B_I$ pair induced a separation of the A_I and B_I subunits, resulting in the wide interface, similar to the bindable-like structure (Fig. 2d, panel C). This emergence of the bindable-like structure is reasonable because the incoming ATP can bind to the more open bindable-like structure.

Fig. 2 Rotation behavior of V_1 -ATPase and main results of example studies. **a** Reaction scheme of the 120° rotation. Each structure for $2_{\text{ATP}}V_1$, $2_{\text{ADP}}V_1$, and $3_{\text{ADP}}V_1$ corresponds to a crystal structure (PDB ID 3VR6, 5KNB, and 5KNC, respectively). **b** Root-mean-square fluctuations (RMSFs) of the A_1B_1 pair for an all-atom MD simulation (red) and coarse-grained (CG)-MD simulations. The deviations are shown in the inset (Isaka et al. 2017). **c** Root-mean-square deviations (RMSDs) of the A_1B_1 pair during a simulation. **d** The time evolution of the rotation angle and typical structures in a CG-MD simulation. The sub-letters A–F represent each snapshot, pointing in the direction of rotation angle progression (Isaka et al. 2017). **e** Summary of the dynamical rearrangements (Isaka et al. 2017)

However, the formation of the bindable-like structure is not the only requirement for the stalk rotation. Steric hindrance must be avoided for the successful rotation. In several CG-MD simulations, the stalk rotation did not occur due to the steric hindrance between the stalk and the B_1 subunit. Namely, the B_1 subunit acts as a gate, and the stalk cannot rotate when the gate is closed. By contrast, in simulations exhibiting successful rotations, the gate was open, and a space between the B_1 and A_{III} subunits was generated (Fig. 2d, panel D). After the emergence of the bindable-like structure, the A_{III} subunit pulled the stalk outward, and then the stalk tilted. The tilt of the stalk was a trigger of the rotation, as discussed later in this review. Here, the tilt of the stalk is observed in the $2_{\text{ADP}}V_1$ crystal structure that was not used in the simulations. As the simulation proceeded, the A_{III} subunit moved further to the outside, and the movement induced the separation of the A_{III} subunit and the B_1 subunit. The separation became large owing to their oppositely oriented movements, resulting in a creation of space, i.e., the open gate. The maximal width of the gate was ~ 28 Å, which was enough to avoid any steric hindrance (Fig. 2d, panel E). Such large openings were not observed in the crystal structure (~ 22 Å). After the passage of the stalk through the gate, the gate was closed, coupled with the closing motion of the A_1B_1 pair from the bindable-like structure to the closed structure. The closed gate prevents the reverse rotation.

Interaction patterns among the AB pairs and the stalk during the rotation were analyzed to gain an understanding of how the dynamical rearrangements of the AB pairs propagate the stalk (Fig. 2e). The penetrated part of the stalk consists of two helices corresponding to the N- and C-terminus, denoted as D_N and D_C , respectively. Before rotation, the A_1 subunit was only in contact with D_N . Following the tilt of the stalk, the interface between the A_1 subunit and the stalk shifted inward. The A_1 subunit approached D_C , coupling with the closing motion of the A_1 subunit. The A_1 subunit then came into contact with both D_N and D_C just before the open gate. After the open gate, the stalk moved into the space and rotated, coupling with the outward motion of the A_{III} subunit and the closing motion of the A_1 subunit, respectively. Finally, the A_1 subunit was only in contact with D_C . Taken together, three events cooperatively contributed to the stalk rotation: the closing motion of the A_1 subunit pushed the stalk, the outward motion of

the A_{III} subunits pulled the stalk, and the open gate, through the rearrangement of the B_1 subunit, generated a space for stalk rotation. Interestingly, just before the open gate, the distance between the A_1 subunit and the stalk was in good agreement with that calculated by the half-closed structure in the $3_{\text{ADP}}V_1$ crystal structure. Actually, in the crystal structure, the A subunit in the half-closed structure is in contact with both D_N and D_C , suggesting that the bindable-like structure undergoes a closing motion upon ATP binding, and the interaction between the A subunit and the stalk changes. An important difference is the adjacent pair of the half-closed structure. As described above, the A_{III} subunit should move outward to avoid steric hindrance and, therefore, it is necessary for the adjacent pair to adopt the open structure. This implies that the conformations of the three pairs in the $3_{\text{ADP}}V_1$ crystal structure do not emerge together during a successful 120° rotation.

In summary, using multiscale MD simulations, cooperative rearrangement motions among the AB pairs were revealed in a CG residue resolution (Isaka et al. 2017). Although nucleotides were not explicitly included in these authors' simulations, binding states could be estimated from conformational changes, allowing the authors to propose a rotation mechanism of a 120° rotation, as follows. ATP hydrolysis and P_i release occur at the $A_{\text{III}}B_{\text{III}}$ pair, and the A_{III} subunit moves outward. Due to the interaction, the A_{III} subunit pulls both the B_1 subunit and the stalk outward, and the motion induces separation of the A_1B_1 pair and a tilt of the stalk. Consequently, the A_1B_1 pair undergoes a conformational change to the bindable-like structure. When ATP binds to the bindable-like structure, the closing motion from the bindable-like to bound structure is induced. Additionally, when ADP release occurs in the $A_{\text{III}}B_{\text{III}}$ pair, the A_{III} subunit moves further outward. Due to the outward motion, the A_{III} and B_1 subunits separate from each other, and then a space between them is created, resulting in the opening of a gate to avoid the steric hindrance between the B_1 subunit and the stalk. Finally, the stalk passes through the gate, and a 120° rotation is achieved. In this mechanism, dynamical rearrangements of the three AB pairs play a crucial role, rather than chemical and structural features of the stalk itself, as in the case of F_1 -ATPase previously described. This point is supported by the single-molecule experiments of F_1 - and V_1 -ATPase (Furuike et al. 2008; Baba et al. 2016). Recently, all-atom analyses have been conducted using the path sampling and free energy calculation techniques (Singharoy et al. 2017). Interestingly, the proposed mechanism in the all-atom model is essentially close to the picture provided in the multiscale MD simulations explained here.

Conclusion

To understand protein functions, a bridging approach between the large-scale dynamics observed in experiments and the

conformational changes at the atomic level is necessary. The use of all-atom molecular dynamics (MD) simulation is one of the most promising approaches because MD simulation is capable of capturing conformational changes obeying the intrinsically structural features. However, the limitation of the timescale of MD simulation still remains a practical problem. To overcome this limitation, the combined use of sampling techniques or coarse-grained (CG) approaches with all-atom MD simulations, termed multiscale MD simulation, is utilized. In this review, we focus on two approaches, each applying to rotary motor protein F_1 - and V_1 -ATPase. The first approach is free energy landscape (FEL) analysis, in which the FEL is calculated by the combined use of the path generation technique and the sampling technique with all-atom MD simulations (Ito et al. 2011; Ito and Ikeguchi 2015). The second approach is CG-MD simulations with the fluctuation matching methodology, in which structural fluctuations of CG-MD are matched to those of all-atom MD simulations (Isaka et al. 2017).

The FEL analyses of F_1 -ATPase have enabled the detailed mechanism of the closing/opening motions of the catalytic subunit (pair) to be determined at atomic resolution (Ito et al. 2011; Ito and Ikeguchi 2015). The motions are mainly achieved by B-helix sliding. Additionally, to connect the closing/opening motions of a subunit pair with dynamical rearrangements in the asymmetrically packed $\alpha_3\beta_3$ subunits, the packing-exchange dynamics has been observed during the stalk rotation (Yoshidome et al. 2011).

Using CG-MD simulations of V_1 -ATPase, cooperatively rearranging motions among the three different AB pairs have been revealed at CG residue resolution (Isaka et al. 2017). Owing to the fluctuation matching methodology, the CG parameters are tuned so that the structural fluctuation around the minimum in CG-MD simulations matches that in all-atom MD simulations. The CG-MD simulations could reproduce a successful 120° rotation with no substeps, and intermediate structures spontaneously emerge in the simulations. Based on the simulated trajectories, the rotation model of V_1 -ATPase was proposed.

Because the computational power is increasing with each passing year, the capability of MD simulations will expand. Multiscale approaches would allow us to tackle very long timescale dynamics, and the improvements of the approach would provide more physicochemically proper pictures, for example, a kinetically proper ensemble given by a Markov state model (Chodera and Noe 2014) or the weighted ensemble approach (Zuckerman and Chong, 2017).

Acknowledgments This work was financially supported by Innovative Drug Discovery Infrastructure through Functional Control of Biomolecular Systems, Priority Issue 1 in Post-K Supercomputer Development from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) to M.I. (Project ID: hp150269, hp160223, and hp170255); by Basis for Supporting Innovative Drug Discovery and Life

Science Research (BINDS) from Japan Agency for Medical Research and Development (AMED) to M.I.; and by RIKEN Dynamic Structural Biology Project to M.I. We further thank collaborators Dr. Yuko Ito (AIST), Dr. Tomotaka Oroguchi (Keio University), Dr. Takashi Yoshidome (Tohoku University), Prof. Nobuyuki Matubayasi (Osaka University), Prof. Masahiro Kinoshita (Kyoto University), Dr. Yuta Isaka (FBRI), Dr. Yuichi Kokabu (MKI), Prof. Ichiro Yamato (Tokyo University of Science), and Prof. Takeshi Murata (Chiba University).

Compliance with ethical standards

Conflicts of interest T. Ekimoto declares that he has no conflict of interest. M. Ikeguchi declare that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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