Published in final edited form as:

J Phys Chem B. 2017 April 20; 121(15): 3502–3514. doi:10.1021/acs.jpcb.6b09350.

Methodology for the Simulation of Molecular Motors at Different Scales

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Abstract

Millisecond-scale conformational transitions represent a seminal challenge for traditional molecular dynamics simulations, even with the help of high-end supercomputer architectures. Such events are particularly relevant to the study of molecular motors — proteins or abiological constructs that convert chemical energy into mechanical work. Here, we present a hybrid-simulation scheme combining an array of methods including elastic network models, transition path sampling and advanced free-energy methods possibly in conjunction with generalized-ensemble schemes to deliver a viable option for capturing the millisecond-scale motor steps of biological motors. The methodology is already implemented in large measure in popular molecular dynamics programs, and can leverage the massively parallel capabilities of petascale computers. Applicability of the hybrid method is demonstrated with two examples, namely cyclodextrin-based motors and V-type ATPases.

Graphical abstract

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Introduction

Molecular motors are nanoscale devices, which harness the free energy from chemical reactions into mechanical work with minimal dissipation. Such motors serve an important purpose in living organisms, driving conformational transitions that regulate a variety of biological processes, which embrace RNA translocation, ATP synthesis and hydrolysis and cytoskeletal transport.¹ Chemists have learned lessons taught by the cell machinery and the principles of energy transduction in biological motors to design and synthesize structurally simpler, yet functionally targeted abiological devices.² These devices have found applications in various areas of molecular recognition, encompassing nanosensors and transducers, which, in turn, can be employed in an automated platform for the synthesis of small molecules, defining an area of frontier research that was awarded the Nobel Prize in Chemistry in 2016. Both biological and abiological motors operate in a thermal bath conducive to free-energy loss owing to fluctuations. As a result, either through evolution or retrosynthetic strategies, these motors are designed to minimize such dissipative losses.

Notable examples of biological motors include **(i)** ATP synthases,^{3–5} which, via rotatory catalysis,^{6,7} synthesize or hydrolyze ATP — the ubiquitous energy source in living organisms — harnessing the free energy from a transmembrane proton gradient, **(ii)** hexameric helicases, in particular Rho-helicases,^{8–10} which, via translational motion, utilize the chemical energy arising from ATP hydrolysis to displace RNA, and **(iii)** dynein and myosin,^{11–13} both key actors in cytoskeletal transport, which, via stepping motion, moves cargo along a microtubule.¹⁴ In the abiological realm, prominent examples include rotaxanes,^{15,16} wherein an external stimulus perturbs the balance in molecular-recognition events,^{17–21} setting in motion the macromolecular ring with respect to the polymer onto which it is threaded, thereby forming the basis of shuttling. They also include molecular pistons,²² wherein combination of complexation and isomerization in a substituted cyclodextrin results in mechanical work entailed by compression–decompression strokes, in the spirit of car engines.

A common feature of biological and abiological motors lies in their function of absorbing energy from a chemical source, converting it into useful mechanical work and dissipating into the sink. Under most circumstances, the energy source stems from reorganization of bonded and nonbonded interactions, while the work underlies conformational transitions, and the sink corresponds to the thermal bath. Chemomechanical coupling in molecular motors is characterized by entangled movements of its components. Not too surprisingly, this coupling is often found to be more intricate in biological motors than in their abiological counterparts,^{23,24} which can be understood by differences in their respective molecular architecture (see Figure 1). Why is it then crucial to dissect the chemomechanical coupling at hand in terms of individual movements resulting in a concerted motion? In biological motors, anomalous motions are implicated in a host of pathologies,^{25–27} which include various forms of cancers, as well as cardiovascular, neurological and reproductive disorders. Conversely, in abiological motors, motion of their structural components can be employed, for instance, as a vehicle for controlled drug delivery;^{28,29} dysfunctional motors are prone to result in poorly targeted capture and release of the therapeutic agent. Optimizing the efficacy

of the molecular motor by identifying the movements at play forms the basis of computer-aided nanodevice design.

At the theoretical level, a detailed picture of motor action by means of computer simulations mandates complete modeling of the energy acquired from the source, the conformational transition and the energy released to the sink. As hinted previously, the energy source arises from the rearrangement of the intra- and intermolecular interactions at play through recognition and association, as well as modification of electronic structures in covalent bond creation and breaking. Under favorable circumstances, a three-dimensional structure of suitable resolution of the motor components is available from crystallography to cryo-electron microscopy experiments, allowing the relevant chemical reaction to be modeled with appropriate accuracy. Alchemical free-energy calculations³⁰ provide a convenient theoretical framework to describe the energy source, either covalently or non-covalently, assuming knowledge of an initial configuration of the chemical partners amenable to reactive trajectories. If such were not the case, one might resort to a combination of molecular docking and sampling strategies to approach the initial energy course configuration.

The intricate nature of the motor action reflected in the entangled movements of its components implies that the conformational transition will follow an equally intricate transition pathway.^{31,32} This pathway can be constructed employing a variety of simulation methods — e.g., molecular dynamics (MD), steered molecular dynamics (SMD),³³ string method with swarms of trajectories (SMST),³⁴ milestoning³⁵ and transition path sampling (TPS)^{36,37} — and combinations thereof. Geometric free-energy calculations³⁰ are then performed to determine the free-energy change along the optimized transition pathway, expected to be the minimum free-energy pathway (MFEP).^{31,32}

In an ideal molecular motor, the free energy produced by the chemical reaction (modeled by means of alchemical free-energy calculations) is anticipated to be consumed entirely by the conformational transition underlying the motion (modeled by geometric free-energy calculations). In practice, however, thermal noise limits the efficiency of the motor through dissipative loss of chemical energy— even though complex biological motors like F₁–ATPase have been shown to operate with a near-100% efficiency.³⁸ It is, therefore, legitimate to ask how the conformational transition is designed to minimize dissipation. In biological motors like Rho-helicase, the dissipative loss is partially contained by dint of noncovalent interactions at the protein-protein interface.³⁹ In fact, the allowable dissipative loss is subservient to the free energy required to drive the conformational transition — e.g., though ATP hydrolysis generates about 8 kcal/mol of energy, only about 1.5 kcal/mol is consumed by Rho-helicase towards RNA translocation by one register. In stark contrast, abiological motors can sometimes consume the entire chemical energy available to perform mechanical work. A cogent example is provided by 6A-deoxy-6A-(N-methyl-3-phenylpropionamido)- β -cyclodextrin binding reversibly 1-adamantanol, resulting in the compression-decompression strokes of a piston with nearly zero dissipative loss.²² While in the latter paradigmatic example and other related, cyclodextrin-based artificial machines the energy of molecular recognition can be almost entirely harnessed into mechanical work,⁴⁰

one should, however, remain cautious and not misconstrue that all abiological motors, in particular those possessing a more complex, sophisticated architecture, are 100% efficient.

The present work builds upon milestone theoretical contributions in the areas of biological^{41–47} and abiological motors,^{48–53} and was made possible by the conjunction of algorithmic advances^{31,32,54,55} and scalable parallel molecular dynamics on petascale architectures.^{56–58} Here, we outline a general, multistep strategy for the computational investigation of biological and abiological motors. The proposed theoretical framework is designed to describe the different legs of the thermodynamic cycle that underlies molecular motor action. After outlining the theoretical underpinnings of the computational strategy, we provide a workflow for modeling chemomechanical coupling in molecular motors. The strategy is then showcased in two concrete examples, namely ADP inhibition of vacuolar V₁–ATPase motor action,^{59,60} and compression–decompression stroke in 6A–deoxy–6A–(N–methyl–3–phenylpropionamido)– β –cyclodextrin motor.²²

Methods

Theoretical Underpinnings

Central to the understanding of how biological and abiological motors operate is the underlying free–energy change that characterizes, on the one hand, the energy source — i.e., the fuel of the motor, and, on the other hand, the conformational transition — i.e., the motor action. These operations are also representative of transitions at multiple timescales, which span a complete motor-action cycle. Broadly speaking, motor-action is accompanied by diffusion of the fuel into the motor, chemical reaction, conformational transition, and diffusion of the product out of the motor to be replenished by new fuel molecules. Normally, the coupling between these events determines their sequence. When coupling is strong and all four events are concomitant, the only possible way of capturing them is within the framework of QM-MD approaches, such as Car-Parinello molecular dynamics.⁶¹ However, coupling between the different motor-action steps is often weak, and the aforementioned events can be discretized in time, i.e., chemical reactions are the fastest, whereas the diffusive binding-unbinding processes are the slowest (rate-determining), and conformational transitions occur at an intermediate rate.³⁹ Within the premises of this discrete chain of events, namely chemical, mechanical, and diffusive, the thermodynamic and kinetic contributions of motor-action ought to be determined with distinct, well-suited approaches. Free–energy calculations in either biological or abiological assays can be arbitrarily distinguished in terms of alchemical and geometrical transformations.³⁰

Fuel Source—Alchemical free–energy calculations exploit the malleability of the potential energy function and the virtually unlimited possibilities of computer simulations to transform between chemically distinct states.⁶² They are, therefore, eminently suitable for the description of the chemical reaction that provides the energy source for motor action. Alchemical transformations require the definition of a progress variable, often referred to as coupling parameter,^{62,63} which connects the end states of the chemical reaction and generally delineates a nonphysical path. Traditionally, variation of the free energy upon reorganization of bonded and nonbonded interactions is determined using a perturbative

approach, i.e., free-energy perturbation (FEP),^{64,65} or gradient-based approach, i.e., thermodynamic integration (TI).⁶⁶ QM/MM calculations can, in principle, model the fuel source with adequate accuracy. However, system size of the biological motors in particular limits the feasibility of these calculations.⁶⁷

Conformational Transition—In stark contrast, geometrical free-energy calculations embrace positional, orientational, and conformational transitions described by a suitably chosen reaction coordinate model, often consisting of a collective variable or combinations thereof. Measure of the free-energy change that underlies these geometric transitions can be achieved employing a host of methods, which can be roughly classified into four distinct categories, namely (i) histogram-based approaches,^{62,63} e.g., umbrella sampling and its multiple variants,^{68–71} (ii) perturbation theory, e.g., FEP,^{64,65} (iii) nonequilibrium work experiments, e.g., SMD simulations³³ in conjunction with the Jarzynski identity,⁷² and, (iv) gradient-based schemes, e.g.,^{62,63} adaptive biasing force (ABF) and related algorithms.^{55,73} One common denominator of these numerical approaches lies in the stratification of the reaction pathway⁷⁴ into a set of intermediate states or windows spanning a range of values of the reaction coordinate model.

It ought to be emphasized at this stage that while these methods have different merits and drawbacks, they also suffer from similar shortcomings, notably in connection with the upstream modeling of the reaction coordinate. It would, therefore, be misleading to assert that one method — or one class of methods, is clearly superior to the others. A number of weaknesses endogenous to geometric free-energy calculations, notably non-ergodicity behaviors, can, however, be addressed cogently by means of a seamless combination of the free-energy method at hand and generalized-ensemble techniques,^{75,76} e.g., bias-exchange umbrella sampling (BEUS)^{58,77} and multiple-walker adaptive biasing force (MW-ABF).^{57,78}

In the BEUS scheme,^{58,77} biasing potentials are exchanged between neighboring windows according to an exchange rule to specifically accelerate sampling of those degrees of freedom most relevant to the transition of interest. In contrast, in the MW-ABF algorithm,^{57,78} a series of walkers explore the free-energy landscape in an independent fashion, exchanging regularly information about the gradient being measured locally. The algorithm can be refined by means of Darwinian selection rules, whereby the most efficient walkers are promoted and spawned at the expense of the least efficient ones.

Minimum Free-Energy Pathway—The concerted movements of motor components that underlie the conversion of chemical energy into mechanical work is captured employing transition path-optimization methods. SMST³⁴ is one such method that refines iteratively a trial transition pathway, referred in this context to as a string,⁷⁹ until the pathway is converged. The string is defined in a high-dimensional space of collective variables by a number of conformations or images, the position of which is updated at each iteration. The average drift applied to each image is estimated from a swarm of short unbiased MD trajectories, shot at the instantaneous position of the image. Each set of unbiased trajectories is followed by a biased equilibration stage, moving the replicas to their updated position.

The iterative process continues until the string converges to an MFEP characterizing the conformational transition.

As has been outlined previously, geometric free-energy calculations require the definition of a reaction coordinate model,^{30,62} which can be provided by such approaches as SMST. Once the converged string is obtained, the free-energy change can be determined using one of the methods enumerated in the last subsection. The missing link between the optimized pathway and the free-energy method is the progress variable or general-extent parameter that tracks conversion between the end-states of the geometric transformation. This progress variable can either be implicit or explicit. In the former case, use is made of geometric variables, i.e., Cartesian or generalized coordinates, to morph between the images of the string. Conversely, in the latter case, a path-collective variable⁸⁰ is defined as a function of the Cartesian coordinates and continuously changes between 0 and 1 as the image morphs between the end-states.

Motor Turnover Rates—So far, we have carefully utilized the vocabulary *reaction coordinate model* to describe the variable along which the free-energy change is determined. This conservative nomenclature stems from the fact that, in general, our knowledge of the actual *reaction coordinate* — a high-dimensional mathematical object that defines the MFEP between the end-states of the transformation, is limited.³⁷ For obvious practical reasons, the reaction coordinate is often modeled in terms of a reduced set of variables of suitable collectivity, connecting the reference and the target states.³⁰ Yet, this choice does not guarantee that the correct transition pathway be followed, which entails that the simulated dynamics does not reflect the actual timescales of the geometric transformation. While this consequence has no bearing on the measured free-energy change by virtue of its path independence, the kinetic properties of interest are prone to be erroneous. It should be clearly understood that a string optimization with swarms of trajectories³⁴ can approach the true reaction coordinate only under the assumption that it encompasses all relevant collective variables. In practice, it is generally recommended to verify a posteriori that the string coincides with the MFEP by means of a committor analysis.^{37,81}

Energy-Conversion Efficiency—Free-energy changes measured from the aforementioned alchemical and geometrical transformations represent, respectively, the energy input into a motor from a fuel source and its subsequent consumption by the motor components to perform mechanical work during the conformational transition. The ratio of these two energy contributions yields the energy conversion efficiency of a molecular motor. Furthermore, from the rate constants determined along the MFEP, one can infer the turnover rate of the motor.⁸² Both the energy conversion efficiency and turnover rate of a molecular motor are estimated from single-molecule experiments. Results from the theoretical strategy presented here are, therefore, readily comparable with experiment. More importantly, the simulations from whence they are obtained provide a detailed picture of the chemomechanical coupling that underlies motor action, the mechanism of which remains only partially understood by experiments.

Modeling Strategy

In this section, we put forward a computational workflow for capturing the action steps of biological and abiological motors, summarized in Figure 2. Towards this end, we will dissect the different contributions of the chemomechanical coupling that eventually leads to spontaneous motor action, and outline how these contributions can be quantified.

Trial Pathway—The reliability of the transition pathway determined using the aforementioned string algorithm,³⁴ and, hence, that of the thermodynamic and kinetic properties inferred from geometric free-energy calculations,³⁰ depend acutely on the quality of the trial pathway — i.e., upstream from string optimization. Optimization of an unphysical trial pathway often results in convergence to functionally irrelevant transitions, which can severely depart from the true reaction coordinate. Thus, construction of a physically meaningful trial pathway is key to the accuracy of the string calculation.

An array of techniques have been developed that supply the guess initial pathway. Targeted MD⁸³ constitutes one such technique, which allows a path to be constructed by minimizing the RMSD between its two end-states. Alternatively, elastic network models (ENM) are designed,^{84,85} employing harmonic spring approximations to provide the lowest potential energy pathway connecting the end-states of the geometric transformation, which represent basins of the potential energy hyperplane.

In certain cases, only one end-state of the transition pathway is known. Under these premises, the other end-state can be constructed employing SMD simulations,³³ steering the relevant degrees of freedom away from the energy minima characterizing the known end-state to an alternate one that represents the other end-state of the pathway. It ought to be understood that this naive approach does not offer any guarantee that (i) another basin will be found, and, (ii) whatever is found corresponds, indeed, to a functionally relevant state.

Monitoring the height of potential-energy barriers in ENM trial pathways, or the magnitude of non-equilibrium work in SMD trial pathways provide a qualitative idea on the functional relevance of the conformations that have been sampled, as well as the sequence of events that delineate the initial pathway. Generally, the lower the height of the potential-energy barrier, or the lesser the magnitude of the work required to induce a transition, the more reliable the initial pathway.³¹ Not too unexpectedly, a higher quality trial pathway converges to the putative reaction coordinate in a smaller number of string iterations.

Pathway Optimization—Notwithstanding proper initiation through a convincingly designed trial pathway, the accuracy of an SMST motor-action pathway is dependent on the choice of the set of collective variables that underlies the string and determines its dimension. As already commented on, SMST with an incomplete set of collective variables converges to unphysical pathways, yielding erroneous kinetic properties. In practice, collective variables are introduced to specifically capture those degrees of freedom that are most relevant to the transition of interest. These collective variables are often constructed by analyzing the structural changes in the trial transition pathway. For example, Cartesian positions of a set of atoms can be chosen as collective variables if, within the initial transition pathway, the root mean-square deviation, or the change in the interaction energy

characterizing this reduced set of atoms is comparable to that of the entire molecular structure. Various combinations of atomic positions in the form of generalized coordinates, e.g., bond length, angles, dihedrals and higher dimensional variables like quaternions, can form collective variables.⁸⁶

Identification of the relevant collective variables allow images equally spaced along the transition path to be defined. Iterations of swarms of biased and unbiased MD simulations are carried out at every image, prefacing reparametrization of the string until it converges to the sought MFEP. Shorter MD trajectories and larger swarms are found to provide richer ensembles than longer MD trajectories with smaller swarms. Invoking thousands of MD trajectories simultaneously, the method is amenable to both serial and parallel implementation on supercomputers. Using an implementation that harnesses the parallel capability of a massively parallel architecture by means of spawned replicas, as is implemented in the popular MD program NAMD,⁵⁶ the total number cores per iteration amounts to $N_{\text{core}} \times N_{\text{traj}} \times N_{\text{image}}$, where N_{core} is the number of cores chosen to perform one MD simulation of the molecular assay at hand, N_{traj} is the number of trajectories per swarm, and N_{image} is the number of images forming the string.

Geometric Free-Energy Calculations—Once the string has converged and coincides with the putative MFEP, the free-energy change along the latter can be determined using methods germane to geometric transformations. Such methods have been introduced in the Theoretical Underpinnings section. Here, we focus on two algorithms, namely BEUS^{58,77} and ABF.^{55,73}

Considering $\xi(s)$ approximately represents the MFEP, and s is its arc-length, then the free-energy change along the path under a stiff spring approximation can be written as:³¹

$$A[\xi(s)] \approx F(s), \quad (1)$$

in which $F(s)$ is, up to an additive constant, the free energy associated with the system

perturbed by biasing potential $U(\xi^t) = \frac{k}{2} [\xi^t - \xi(s)]^2$. $F_i = F[\xi(s_i)]$ can be estimated, up to an additive constant, by solving iteratively the equations:^{87,88}

$$e^{-\beta F_i} = \sum_t w^t e^{-\beta U_i(\xi^t)}. \quad (2)$$

Here, w^t represents the unnormalized weight of configuration $\mathbf{x}^t$, and can be estimated via:⁸⁷

$$w^t = \left\{ \sum_i T_i e^{-\beta [U_i(\xi^t) - F_i]} \right\}^{-1} \quad (3)$$

where, T_i is the number of samples collected for image i of the string. Thus, one can estimate $A[\xi(s)]$ in terms of both $\{w^t\}$ and $\{F_i\}$ by solving equations (2) and (3) iteratively.

In order to estimate $F(s)$ numerically, the BEUS scheme can be employed. The mixing of the replicas in BEUS guarantees continuity in the sampling of a conformational tube that embraces the string,⁸⁹ thereby yielding a more reliable free-energy profile for the process than ordinary US. With appropriate reweighting, the potential of mean force (PMF) can be reconstructed in the collective-variable space wherein the string is defined, under the assumption of adequate sampling. One of the techniques used for this purpose is nonparametric reweighting, which assigns a weight to each individual conformation sampled.^{31, 90} A variant of the weighted histogram analysis method (WHAM)⁹¹ can be utilized towards this end and the PMF can then be reconstructed in terms of any arbitrary measurable quantity.

As has been mentioned previously, a possible alternative approach to BEUS is MW-ABF,^{57, 78} wherein a number of walkers explore the free-energy hyperplane concomitantly in the course of an unconstrained MD simulation, exchanging periodically information on the gradient of the free energy. When applied, the time-dependent biasing force, $\nabla \tilde{A}$, yields a Hamiltonian bereft of an average force acting along $\xi(s)$. It follows that all the values spanned by $\xi(s)$ are sampled with an equal probability, under the usual assumption of timescale-separation. In stark contrast with probability-based methods like US and its variants, a purely local estimate of the gradient is utilized, allowing the biasing force to be updated continuously. Furthermore, contrary to BEUS, MW-ABF would require an explicit path-collective variable⁸⁰ inferred making use of the optimized string.

Kinetic Modeling—One attractive feature of motor modeling lies in the possibility to compute turnover rates, which can be readily compared with experiment. Access to this quantity surmises knowledge of the position-dependent diffusivity, $D(s)$, along the MFEP, together with the PMF, the determination of which has been outlined above. Although there are a number of avenues for the estimation of $D(s)$,^{92–94} one promising approach relies on Bayesian inferences and may be viewed as the inverse solution of the Smoluchowski equation,⁹⁵ which supplies consistent estimates of $F(s)$ and $D(s)$, based on the trajectory obtained from a biased simulation. Under the stringent assumption of a diffusive regime, the motion is propagated using a discretized Brownian integrator, whereby the trajectory can be seen as a series of discrete hops of finite time. The probability of the complete trajectory given the parameter of interest, namely $D(s)$, is the product of the probabilities at each step. Using Bayes theorem, one can then infer the probability of $D(s)$ given the trajectory. Put together, finding the optimal position-dependent diffusivity becomes a maximum-likelihood problem of finding a parameter that maximizes the posterior probability, which can be handled by generating a Markov chain of states, employing a Metropolis-Hastings algorithm. From the knowledge of $F(s)$ and $D(s)$, one can then estimate the mean-first-passage time⁹⁶ — or the inverse of the rate constant, between the end-states of the string.

Alchemical Free-Energy Calculations—So far, we have addressed how the conformational transition of motor action, that is its mechanical component, ought to be handled computationally. To complete the chemomechanical coupling, we now outline the treatment of the chemical component, the fuel source of the motor. The latter by and large consists of a chemical reaction involving the reorganization on bonded and nonbonded

interactions, e.g., ATP hydrolysis, substrate binding and release. Strictly speaking, rearrangement of electronic structures would require specific quantum–mechanical calculations to capture bond creations and ruptures.⁶² In practice, however, due to the overwhelming cost of such computations, in particular for large biological objects, one resorts to molecular–mechanical approximations, which is warranted by two important considerations. First, the timescale separation of the conformational transition and the chemical reaction allows the two to be handled independently. Second, whereas the choice of the reaction path is crucial in the modeling of the conformational transition, it is clearly less so for the chemical reaction, which is a corollary of the first consideration. Besides, our interest lies chiefly in the end states of the chemical reaction — not its actual pathway.

Modeling of the chemical reaction can be performed using alchemical free–energy calculations,³⁰ in which the reference state, i.e., the reactant, is transformed into the target state, i.e., the product, modifying the potential energy function. One common theoretical tool towards this end is perturbation theory,^{62,63} whereby the target state can be seen as a perturbation of the reference state. In practice, as a way to reduce the systematic error in FEP calculations,^{64,65} the path connecting the two end states is broken into nonphysical intermediates separated by incremental perturbations. Furthermore, in order to minimize the variance of the calculation, the transformation is carried out bidirectionally between the reactant and the product,⁹⁷ and the statistical data accrued in the forward and the backward directions is combined to yield a maximum–likelihood estimator of the free energy, referred to as Bennett acceptance ratio.⁹⁸

The reorganization of bonded and nonbonded interactions that fuels motor action is often accompanied by significant variations of configurational entropy, which are not easily captured in MD simulations — e.g., association of a flexible substrate with its host, like ATP binding an ATPase. A rigorous theoretical framework has been proposed to address this limitation of MD and relies upon the introduction of a series of geometric restraints enforced as the substrate is being coupled or decoupled reversibly from its environment.^{99–101} Addition of such geometric restraints corresponds to a loss of conformational, orientational and positional entropy, which ought to be quantified in separate alchemical free–energy calculations, wherein the force constant of the biasing harmonic potential is gradually scaled reversibly from its nominal value to zero.

To summarize, we have put forth a practical workflow for the modeling of motor action, embodied in Figure 2, which yields the chemical and the mechanical contributions to the chemomechanical coupling. The ratio of these two contributions quantifies the energy–conversion efficiency of the molecular motor.⁸²

Computational Details

Cyclodextrin–Based Motor—In this first application of the methodology proposed for the investigation of molecular motors, the compression–decompression stroke of a prototypical abiological motor consisting of a β -cyclodextrin derivative, namely 6A–deoxy–6A–(N–methyl–3–phenylpropionamido)– β -cyclodextrin,²² binding reversibly 1–adamantanol is simulated with the assumption that the entire chemical free energy is converted into mechanical work. Figuratively speaking, the aryl moiety of the motor can be

viewed as the piston, whereas the cyclodextrin would correspond to the cylinder. The compression stroke arises from the binding of the aryl moiety by the cavity of the macrocycle cyclodextrin and is triggered by the extraction of the substrate 1-adamantanol in the proper solvent condition — a competition between intra- and intermolecular complexation. It is shown to occur with the Z-isomer of the amide group to which the aryl moiety, i.e., the piston, is attached (see Figure 3). Conversely, the decompression stroke results from the addition of the substrate, and occurs with the E-isomer of the amide group. Reversible binding of the guest, therefore, directly modulates the Z/E isomer ratio of the host.

In stark contrast with larger, more complex biological motors, modeling the compression–decompression stroke in A–deoxy–6A–(N-methyl–3-phenylpropionamido)– β –cyclodextrin is straightforward and does not require preliminary TPS calculations to approximate the MFEP. The reaction pathway for the two isomers can be described in terms of a simple projection of the vector that connects the center of mass of the substrate to that of the β –cyclodextrin cavity. In the case of the Z-isomer, the aryl moiety is initially ensconced inside the macrocycle, whereas for the E-isomer, it stretches outside of the host. The molecular assembly consisted of the motor and the substrate in a bath of about 4,600 water molecules. The MD simulations were performed in the isobaric–isothermal at 1 atm and 303.15 K, using NAMD⁵⁶ with the CHARMM27,¹⁰² CSFF¹⁰³ and CGenFF¹⁰⁴ potential energy functions. Details of the simulations can be found in reference 52.

ATP Synthase—The second MD study reported here is based on a crystal structure of V_1 –ATPase from *Enterococcus hirae* (PDBID: 3VO6).¹⁰⁵ For simulation purposes, the artificial ATP-mimetics, namely AMP-PNP, which are employed as inhibitors for isolating crystals, are replaced by real ATP molecules in the bound states (**b**) and in the vicinity of the empty state (**e**), and ADP and an inorganic phosphate (modeled as $H_2PO_4^-$) in the tight state (**t**) (Figure 4). Structural analyses²⁶ have demonstrated that non-hydrolyzable AMP-PNP can successfully mimic the ATP and ADP + Pi binding states in F-type ATPases; different ATP analogues produce in fact similar binding conformations.²⁶

The two ATP- and one ADP-Pi-bound V_1 (A_3B_3)–ring was solvated respectively in a water box of size $170 \text{ \AA} \times 170 \text{ \AA} \times 170 \text{ \AA}$ with 150 mM NaCl. The simulation system size is about 0.36 M atom large. After a 4000–step energy minimization, the system was heated in the isobaric–isothermal ensemble (1 atm) to 300 K during 50 ps, employing harmonic positional restraints on heavy atoms with a $1 \text{ kcal/mol/\AA}^2$ spring constant. Maintaining the same harmonic potentials, a 1 ns equilibration was carried out in the isobaric–isothermal ensemble (1 atm at 300 K), followed by a 4 ns canonical–ensemble simulation, gradually decreasing the spring constant to zero during the latter stage. All MD simulations were performed using NAMD⁵⁶ with the CHARMM27 force field¹⁰² and CMAP corrections.¹⁰⁶

Results

Compression–Decompression Strokes of a Paradigmatic Abiological Motor

In this first illustration of a rudimentary, cyclodextrin-based abiological motor, the transition coordinate is well identified, thereby obviating the need for tedious search of the most

probable MFEP. Here, action of the piston is intimately coupled to the binding of the substrate to the cavity of 6A–deoxy–6A–(N–methyl–3–phenylpropionamido)– β –cyclodextrin, notably favorable van der Waals interactions, which supplies the chemical energy of the motor. This nearly ideal scenario is, however, not representative of simple, abiological motors, for which the conformational transition may consist of more entangled movements. For instance, in certain rotaxanes, e.g., a benzylic amide macrocycle threaded onto a fumaramide moiety capped by two 1,2–dibenzyl ethyl groups,¹⁰⁷ shuttling between stations cannot be described in terms of a mere translation of the threaded ring, but ought to be viewed instead as a superimposition of translational and rotatory motions, accompanied by *synanti*-isomerization of amide groups.⁵³ Under these premises, careful identification of a suitable set of collective variables, appropriate for the description of the underlying contributions to the overall conformational transition is necessary. Post–hoc assessment of the model reaction coordinate, for instance, by means of a committor analysis,^{37,81} is recommended, in particular if the kinetic properties of the abiological motor are sought.

The PMFs, $R(\xi)$, delineating the reversible binding of 1–adamantanol to the cavity of 6A–deoxy–6A–(N–Methyl–3–Phenylpropionamido)– β –cyclodextrin for the Z– and the E–isomers of the amide moiety are depicted in Figure 5. From the onset, it can be seen that, irrespective of the isomer, complexation of the substrate by the macrocycle corresponds to a metastability on the free–energy landscape. The minimum of the one–dimensional free–energy profile for the E–isomer is, however, somewhat deeper than that for the Z–isomer. The difference of approximately 2.2 kcal/mol between the well depths can be understood in terms of more favorable interactions of 1–adamantanol with the N–methyl–3–phenylpropionamido group forming the piston for the E–isomer, in particular for the value of the transition coordinate corresponding to the free–energy minimum. Under the assumption of proper averaging of all other relevant degrees of freedom and considering that association follows a cylindrical symmetry, the binding constant of the substrate to the cavity of the cyclodextrin can be expressed as,¹⁰⁸

$$K_{\text{eq}} = 2\pi \int_0^{\xi_{\text{max}}} d\xi \xi e^{-\beta F(\xi)} \quad (4)$$

where ξ_{max} stands for the cylindrical radius separating the bound and the unbound states of the substrate with the macrocycle. $\beta = (k_B T)^{-1}$, where k_B is the Boltzmann constant and T is the temperature. The standard binding free energy is $G^\circ = -k_B T \log(K_{\text{eq}} C^\circ)$, where C° is the standard concentration, i.e., $1/1661 \text{ \AA}^3$. Assuming that all the chemical energy is consumed by the piston action, the chemomechanical work, δW , can be determined as the difference between the binding free energies for the compression and the decompression strokes — in other words for the binding processes featuring the Z– and the E–isomers.⁵² In light of the free–energy profiles of Figure 5, the equilibrium constant for the Z– and the E–isomers is found to be equal to 8.76×10^6 and $2.15 \times 10^8 \text{ M}^{-1}$, respectively. The hemomechanical work, i.e., the difference in the corresponding binding free energies, simplifies to $\delta W = -\beta^{-1} \ln(K_{\text{eq}}^Z / K_{\text{eq}}^E)$ and is equal to +1.9 kcal/mol, in good agreement with the experimental measurement of +1.4 kcal/mol.²²

Pathway of ADP Inhibition in Vacuolar V_1 -ATPase Motor Action

A second example showcasing the simulation protocol presented here is provided by the pathway of ADP inhibition in the hexameric ring of the molecular motor V_1 -ATPase.⁵⁹ Vacuolar ATPases, or V-ATPases, are thought to originate from an ancestral enzyme in common with the more ubiquitous F-ATPases.⁶⁰ The main function of V-ATPases in eukaryotes is the transport of protons across the membrane, using the energy produced by ATP hydrolysis. V-ATPases also catalyze ATP synthesis, exploiting the energy of the proton flow in certain eubacteria, e.g., *Thermus thermophilus*.

Boyer developed a binding-change model,^{4,6,7} which postulates that ATP synthesis is coupled with a conformational change in the ATP synthase generated by rotation of its central stalk. Despite the success of Boyer's model and its various extensions based on crystallographic and single-molecule experiments, a comprehensive allosteric pathway elucidating the so-called chemomechanical coupling^{105,109} between the chemistry of ATP synthesis or hydrolysis and the mechanics of V_1 motor action has remained hitherto elusive.

Here, combining anisotropic network models,^{84,85} SMST³⁴ and BEUS simulations,^{58,77} we present a detailed description of the ATP hydrolysis-driven rotatory step in the A_3B_3 ring of V_1 -ATPase when the hydrolysis product, ADP-Pi, is not released from the reaction site. The A_3B_3 ring includes three ATP/ADP binding sites referred to as empty (**e**), bound (**b**) and tightly bound (**t**) — see Figure 4. The occupancy of these sites is strongly correlated to the conformations of the protein, denoted respectively A^eB^e , A^bB^b , and A^tB^t , according to the binding interface they form during the ATP hydrolysis cycle. The initial and final states of the conformational transition in A_3B_3 to be probed here differ by a 120° rotation of the **e**, **b** and **t** states. This rotation, coined conformational rotation, carries a near zero moment of inertia.

Elastic Network Model of the V_1 -Ring—Conformational rotation of the A_3B_3 ring is examined employing anisotropic network models (ANM)¹¹⁰ to build a trial pathway, which will be subsequently refined by means of string calculations. Seven ANM pathways were constructed at C_α -resolution between the end states of the A_3B_3 ring (Figure 6). All three protein dimer states are allowed to change simultaneously in the first step, i.e., (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^tB^t). Next, two protein dimer states change simultaneously, i.e., (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^bB^b), (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^eB^e$, A^eB^e , A^tB^t) and (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^tB^t , A^bB^b). Last, only one dimer state changes at a time, i.e., (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^tB^t , A^bB^b), (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^eB^e$, A^eB^e , A^bB^b) and (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^eB^e$, A^tB^t , A^bB^b). The product of the pathway endowed with the lowest energetic barrier among the seven distinct ANM pathways, i.e., (A^eB^e , A^eB^e , A^bB^b), was subjected to two further transitions, namely (A^eB^e , A^eB^e , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^bB^b) and (A^eB^e , A^eB^e , $A^bB^b \rightarrow A^eB^e$, A^eB^e , A^tB^t). The former pathway was found to exhibit a lower barrier. Thus, the final step involves a (A^bB^b , A^eB^e , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^tB^t) transition.

Altogether, the pathway connecting (A^eB^e , A^tB^t , A^bB^b) to (A^bB^b , A^eB^e , A^tB^t) follows the sequence of transitions (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^eB^e$, A^eB^e , A^bB^b) $\rightarrow$ (A^eB^e , A^eB^e , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^bB^b) $\rightarrow$ (A^bB^b , A^eB^e , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^tB^t). This pathway is

characterized by a sum of 30 [see Figure 6(c)] + 25 [see Figure 6(d)] + 20 [number of models along the (A^bB^b , A^eB^e , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^tB^t) pathway] = 75 models in total. The lowest potential energy pathway was resolved at full-atomic detail via TMD simulations.⁸³ These simulations bias the C_α structure of state (A^eB^e , A^tB^t , A^bB^b) onto each one of the 75 models along the ANM pathway, while letting all other atoms in the structure relax, thereby providing an all-atom trial pathway between (A^eB^e , A^tB^t , A^bB^b) and (A^bB^b , A^eB^e , A^tB^t). This ANM-based TMD pathway is discretized into 100 equally distant images and optimized subsequently via string calculations,³⁴ as described in the next section, in order to obtain the MFEP for the conformational rotation of the A_3B_3 ring.

Collective-Variable Space—Due to the high dimensionality of the movement causing the (A^eB^e , A^tB^t , A^bB^b) $\rightarrow$ (A^bB^b , A^eB^e , A^tB^t) transition, we had to define the reaction-coordinate model, $\xi(s)$, through a systematic computational procedure, as the large number of collective variables necessary for its representation cannot admittedly be hand-picked. The set of collective variables suitable for the problem at hand should describe the dominant changes that arise during the (A^eB^e , A^tB^t , A^bB^b) $\rightarrow$ (A^bB^b , A^eB^e , A^tB^t) transition. These collective variables are identified to be the positions of key residues pertaining to the six subunit-subunit interfaces of the A_3B_3 trimer. These residues are found to be major contributors to changes in the interface energy, as well as to changes in the overall RMSD observed in the ANM pathways. Eventually, $393 \times 3 = 1,179$ Cartesian coordinates were identified as collective variables (see Figure 7).

String Calculation—Ten replicas of 0.35-ns long MD simulations were performed for each one of the 100 images geometrically restrained to their respective conformational state of the 1,179 Cartesian coordinates. Toward this end, a strong harmonic potential was enforced with a force constant of 50 kcal/mol/Å². A swarm of short trajectories, namely 20×25 ps, was shot subsequently from each image, and the image evolved according to the average drift of the swarm during each iteration.³⁴ At the end of the latter, a reparametrization of the string ensured an equidistant distribution of the images. The pathway was, thus, optimized iteratively until it converged to the most probable transition path, which is an approximation of the MFEP. A stable solution of the transition pathway for the V_1 -ring was obtained within 50 iterations.

The converged string trajectory reveals a distinct series of events that follows ATP hydrolysis. First, A^tB^t becomes A^eB^e , the latter still featuring a bound ADPPi moiety. This transition prefaces binding of an ATP into the neighboring e site. At this stage, all the three sites of the A_3B_3 -ring are occupied by a bound ATP. Finally, the third site, previously in a **b** conformation, i.e., in the absence of incoming ATP, undergoes an A^bB^b to an A^tB^t conformational transition, thereby completing the (A^eB^e , A^tB^t , A^bB^b) $\rightarrow$ (A^bB^b , A^eB^e , A^tB^t) transition.

BEUS Calculation—The BEUS simulations^{58,77} for the putative MFEP were performed employing the aforementioned 1,179 Cartesian coordinates restrained to the position of the images along the optimized string. To ensure sufficient window overlap in the biased simulations, the number of images was increased to 200, i.e., twice the number employed in the string calculation. An exchange of biases was attempted every 1 ps between adjacent and

circumjacent images — i.e., images i and either $i-1$ or $i+1$, and images i and either $i-2$ and $i+2$, in an alternated fashion. Ten replicas per image were employed in 8-ns long BEUS simulations. A force constant of 2 kcal/mol/Å² per atom was employed to restrain geometrically the atomic positions in the course of the free-energy calculations, which results in comparable exchange rates between neighboring windows, ranging typically from 29% to 40%. A simulation of a total of 10 replicas/image $\times$ 200 images $\times$ 8 ns/replica = 16 μ s was performed to construct the one-dimensional free-energy profile along the (A^eB^e, A^tB^t, A^bB^b) $\rightarrow$ (A^bB^b, A^eB^e, A^tB^t) transition pathway. The statistical data accrued in the course of this simulation was employed to construct the relevant histograms and subsequently infer the underlying free-energy change, using the non-parametric reweighting scheme outlined above.⁹⁰

Interpretation of the Free-Energy Change—Overall, the net free-energy change along the putative MFEP is found to be endothermic by +2.2 kcal/mol, implying that the pathway is thermo-dynamically unfavorable (Figure 8). The primary free-energy barrier, about 4.0 kcal/mol high, arises from the ATP binding step, i.e., the second step in the chronology of events underlying the (A^eB^e, A^tB^t, A^bB^b) $\rightarrow$ (A^bB^b, A^eB^e, A^tB^t) transition. This barrier reflects a reorganization of the gatekeeper, R350, residue lining the B subunit, which allows ingress of ATP into its binding pocket in the A subunit.

Both thermodynamic quantities, i.e., the net free-energy change and the barrier, bear significant biological relevance. It has been suggested by crystallographic studies¹⁰⁵ that opening of the tight A^tB^t interface after ATP hydrolysis into an empty A^eB^e interface promotes opening of the neighboring A^eB^e interface even further, allowing it to bind an incoming ATP by facilitating isomerization of the gatekeeper R350 residue. However, presence of the hydrolysis product, ADP·Pi, inside the first A^eB^e interface precludes allosteric opening of the second one. Consequently, the incoming ATP associates only partially to its binding pocket at the A^eB^e interface. Furthermore, presence of bound ATP in all three binding pockets, as was observed in the present trajectory, morphs the A₃B₃ ring into a more symmetric state than when only two of the pockets are occupied. Such symmetric states are expected to engender deleterious elastic stress, making the pathway overall unfavorable. We, therefore, conclude that release of the reaction product from the t site allows thermodynamically favorable binding of ATP in the neighboring e site. In agreement with single-molecule experiments,¹⁰⁹ presence of bound ADP·Pi inhibits ATP binding and subsequent ATP hydrolysis-driven activity of V₁ ATPase. Independent simulations reveal that the ADP·Pi release step takes up to 2.5 ms.⁴⁴ Noting that the average ATP hydrolysis rate of V-type ATPases is approximately 300 sec⁻¹,¹¹¹ the conformational transition should take no more than 0.5 ms. Turning to the expression of the mean first passage time,⁹⁶

$$\bar{\tau}_{F \rightarrow P} = \int_{\xi_A}^{\xi_B} d\xi \frac{\int_{\xi_A}^{\xi} d\xi' \exp(-\beta F(\xi'))}{D(\xi) \exp(-\beta F(\xi))}. \quad (5)$$

with the stated value of $\bar{\tau}_{FP} = 0.5$ ms, energy data from Figure 8, and numerically integrating from image index $\xi_A = 0$ to $\xi_B = 200$, a position-independent diffusion coefficient of $3.8 \times 10^{-8} \text{ cm}^2 \text{ sec}^{-1}$ is found necessary to overcome the kinetic barrier of 4 kcal/mol and sustain the biologically relevant rate of ATP hydrolysis. Such a diffusion coefficient is characteristic of lateral lipid diffusion in the cell membrane,¹¹² which is untenable at ligand-bound protein-protein interfaces. Consequently, a barrier resulting from the ADP-inhibited state will delay the overall rate of ATP activity. In contrast, a conformational transition following the ADP-Pi release is known to bring the barrier down significantly,³⁹ allowing a biologically sustainable rate of ATP hydrolysis.

Conclusion

Modeling of molecular motors represents a daunting challenge for brute-force MD simulations, even in the petascale computing era. The primary obstacle remains simulating intricate phenomena that span the millisecond timescale, which would still be possible for simple motors formed by small molecules such as cyclodextrins¹⁷ or cyanostars,¹¹³ but would become rapidly impossible for more complicated biological motors like ATP synthase, Rho-helicase, dynein or myosin. The hybrid approach presented here, combining an array of methods including ENMs,^{84,85} TPS^{34,37,79} and advanced free-energy methods^{57,58,77} — possibly in conjunction with generalized-ensemble schemes,^{75,76} offers a viable option for capturing the millisecond-scale motor steps of biological motors. The individual methods are, for the most part already implemented in popular MD programs, and can leverage the massively parallel capabilities of petascale computer architectures with molecular assemblies in excess of 1M atoms.

Applicability of our hybrid scheme is subservient to the nature of the molecular motor being investigated. If the reaction coordinate describing the motor-action steps is simple — even intuitively obvious, then a combination of SMD³³ with straightforward US⁶⁸ or BEUS,^{58,77} is expected to capture all the biologically relevant features of the motor. However, one often faces the burdensome task of determining a reasonable model of the reaction coordinate, and the choice of a consistent set of collective variables, prior to simulating the nonlinear conformational transitions that underly motor action, e.g., as cogently illustrated with the example of ATP synthase. Such a scenario would necessitate invocation of almost all the rungs outlined in our hybrid strategy, i.e., choice of a trial pathway and path optimization as a preamble to free-energy and rate computations.

The main limiting caveat of the proposed strategy lies in the availability of both end states of the transition of interest. In the event the alternate state is not structurally characterized, it will have to be predicted, using, for instance, bioinformatics-driven modeling approaches prior to addressing the transition path. Information on intermediates in the form of crystal or electron microscopy structure, even sparser biochemical data, such as key mutations or cross-links, often cross-validate the detailed computational predictions. Beyond its application to molecular motors, showcased here, the current approach is broadly applicable to sampling rare events in the realm of transmembrane ion transport,³² cytoskeletal trafficking^{11–13} and host-pathogen interactions.^{25,26}

Acknowledgments

The present contribution is dedicated to Klaus Schulten (1947–2016), whose visionary developments in parallel molecular dynamics allow today biological motors to be simulated over realistic timescales by means of petascale computing. The authors are indebted to Mahmoud Moradi, Wensheng Cai and Peng Liu for stimulating discussions. The research reported here has been supported by the National Institute of Health through Grants 9P41GM104601 and R01-GM067887-11, and the National Science Foundation through Grants MCB1157615 and PHY1430124. The authors also acknowledge supercomputer time on Stampede provided by the Texas Advanced Computing Center (TACC) at the University of Texas at Austin through Extreme Science and Engineering Discovery Environment (XSEDE) Grants XSEDE MCA93S028. This research used resources of the Oak Ridge Leadership Computing Facility at the Oak Ridge National Laboratory, which is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC05-00OR22725. The authors are also thankful to the Centre Informatique National de l'Enseignement Supérieur (CINES) and the Grand Équipement National de Calcul Intensif (GENCI) for generous provision of computer time. C.C. acknowledges support from the Fonds Européen de Développement Régional (FEDER). A.S. is grateful for financial support from the Beckman Foundation, and NSF through the Center for Physics of Living Cells.

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Figure 1.

Example of a biological and an abiological motor. The biological motor (A) is the vacuolar V_0V_1 -ATPase of *Enterococcus hirae* shown here as a transparent surface. The chemical energy arising from the sequential binding and hydrolysis of ATP at the interface of the three AB subunits that form the A_3B_3 ring of the V_1 -ATPase rotary motor (highlighted in red and yellow), is transformed into mechanical work causing the central stalk to rotate against the A_3B_3 ring. The torque being created is then propagated to the lower V_0 -ATPase rotor. The abiological motor (B) consists of a simple substituted macrocycle, 6A-deoxy-6A-(N-methyl-3-phenylpropionamido)- β -cyclodextrin, wherein the cavity of the cyclodextrin forms the cylinder of the motor and the N-methyl-3-phenylpropionamido moiety, which can insert into the latter, forms the piston. The motor is fueled by 1-adamantanol, which can bind the cyclodextrin and, thus, competes with the N-methyl-3-phenylpropionamido moiety. Reversible binding of the substrate corresponds to the compression-decompression strokes of the motor.

Figure 2.
Computational workflow for the modeling of molecular motors.

Figure 3. Thermodynamic cycle for the compression–decompression stroke in an abiological motor formed 6A–deoxy–6A–(N–methyl–3–phenylpropionamido)– β –cyclodextrin interacting with 1–adamantanol. The upper binding process corresponds to the Z–isomer of the piston amide group and the lower binding process to its E–isomer. K_{eq}^Z and K_{eq}^E are the respective equilibrium constants for the latter binding processes.

Figure 4. A₃B₃ ring of vacuolar V₁-ATPase featuring the three states of the AB interface — empty (e), bound (b) and tightly bound (t). Closeup of the t state, wherein ADP·Pi is tightly bound to the protein.

Figure 5. One-dimensional free-energy profiles characterizing the compression-decompression stroke in an abiological motor formed 6A-deoxy-6A-(N-methyl-3-phenylpropionamido)- β -cyclodextrin binding reversibly 1-adamantanol for the Z- (solid line) and E-isomer (dashed line) of the host. The transition coordinate, ξ , is the projection of the vector connecting the center of mass of the guest to that of the host.

Figure 6.

Energy changes from Anisotropic Network Model (ANM)-predicted pathways of conformational transitions in the V_1 -ring (A_3B_3) complex are employed to screen the most probable sequence of events that connect R^* to F^* . (a) First, all the three protein states are allowed change simultaneously in an ANM pathway: (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^tB^t). (b) Second, two protein states change simultaneously during ANM: (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^bB^b), (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^tB^t , A^tB^t), (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^eB^e$, A^eB^e , A^tB^t). (c) Third, only one state changes at a time: (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^bB^b$, A^tB^t , A^bB^b), (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^eB^e$, A^eB^e , A^bB^b), (A^eB^e , A^tB^t , $A^bB^b \rightarrow A^eB^e$, A^tB^t , A^tB^t). (d) Product from the pathway with lowest barrier among the seven ANM pathways, (A^eB^e , A^eB^e , A^bB^b), was subjected to two further transitions (A^eB^e , A^eB^e , $A^bB^b \rightarrow A^bB^b$, A^eB^e , A^bB^b) and (A^eB^e , A^eB^e , $A^bB^b \rightarrow A^eB^e$, A^eB^e , A^tB^t); the former pathway exhibits a smaller barrier.

Figure 7.

Construction of the reduced space for the string simulations of the V₁-ring complex as well as the V₁-rotor. (a) The nucleotide-containing AB interface is colored by interaction energies showing residues, in green, within a cutoff of 10 Å that contribute most intensely to the interface interactions. (b) The same set of residues capture the major RMSD changes within a tentative R* to F* pathway, thus, implying their significance in deriving a reduced description that probes the R* to F* conformational transition; RMSD is computed with respect to the the thermal-average F* structure. 84 residues are derived from the nucleotide-binding AB interface. An additional 47 residues are derived from the non-nucleotide binding interface. In total, the C-alpha atoms from each of these $3 \times (84+47)$ residues are considered to construct the $3 \times (84+47) \times 3 = 1179$ dimensional reduced space. The A subunit residues included in the subspace are number 7 8 9 10 11 12 54 55 56 57 58 59 60 61 62 83 85 91 92 94 95 101 102 103 104 105 233 234 235 260 261 262 263 264 265 266 267 268 270 271 293 294 295 296 297 298 299 300 333 336 337 340 343 344 346 347 352 353 392 393 from the chains A, B and C in the PDB file of V₁-rotor, and similarly for the B subunit are residue number 23 24 25 26 27 46 47 48 49 76 114 115 116 117 118 119 120 121 122 123 124 144 145 146 266 267 269 270 275 276 278 279 282 283 286 287 289 317 320 321 322 323 324 350 351 from chains D, E and F.

Figure 8.

One-dimensional free-energy profile of the product ADP·Pi-inhibited pathway of $V_1 A_3 B_3$ ring structural transitions. The positive net free-energy change highlights an endothermic reaction and implies that rotation is thermodynamically unfavorable in the presence of products bound to the empty site. The initial, the final and the two intermediate structures are labeled by their image number, i.e., 0, 20, 60 and 200.