

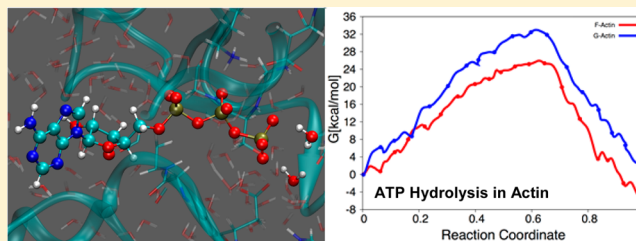
Simulating Protein Mediated Hydrolysis of ATP and Other Nucleoside Triphosphates by Combining QM/MM Molecular Dynamics with Advances in Metadynamics

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S Supporting Information

ABSTRACT: The protein mediated hydrolysis of nucleoside triphosphates such as ATP or GTP is one of the most important and challenging biochemical reactions in nature. The chemical environment (water structure, catalytic metal, and amino acid residues) adjacent to the hydrolysis site contains hundreds of atoms, usually greatly limiting the amount of the free energy sampling that one can achieve from computationally demanding electronic structure calculations such as QM/MM simulations. Therefore, the combination of QM/MM molecular dynamics with the recently developed transition-tempered metadynamics (TTMetaD), an enhanced sampling method that can provide a high-quality free energy estimate at an early stage in a simulation, is an ideal approach to address the biomolecular nucleoside triphosphate hydrolysis problem. In this work the ATP hydrolysis process in monomeric and filamentous actin is studied as an example application of the combined methodology. The performance of TTMetaD in these demanding QM/MM simulations is compared with that of the more conventional well-tempered metadynamics (WTMetaD). Our results show that TTMetaD exhibits much better exploration of the hydrolysis reaction free energy surface in two key collective variables (CVs) during the early stages of the QM/MM simulation than does WTMetaD. The TTMetaD simulations also reveal that a key third degree of freedom, the O–H bond-breaking and proton transfer from the lytic water, must be biased for TTMetaD to converge fully. To perturb the NTP hydrolysis dynamics to the least extent and to properly focus the MetaD free energy sampling, we also adopt here the recently developed metabasin metadynamics (MBMetaD) to construct a self-limiting bias potential that only applies to the lytic water after its nucleophilic attack of the phosphate of ATP. With these new, state-of-the-art enhanced sampling metadynamics techniques, we present an effective and accurate computational strategy for combining QM/MM molecular dynamics simulation with free energy sampling methodology, including a means to analyze the convergence of the calculations through robust numerical criteria.



INTRODUCTION

Nucleoside triphosphates (NTP) contain a nucleoside bound to three phosphate groups. The hydrolysis of NTPs such as ATP or GTP by proteins is one of the most important reactions in biology. It provides energy to biochemical functions that are of great importance to life and plays essential signaling functions. For example, adenosine triphosphate (ATP), one of the most important NTPs, is often considered as a “molecular unit of currency” in intracellular energy transfer¹ and plays essential roles in biological processes such as synthesis of proteins and membranes, movement of the cell, cellular division, etc.^{2,3} Another important NTP molecule, guanosine triphosphate (GTP), is vital to cell signaling, cell motility, protein synthesis and translocation, and more.^{4–6} Due to the importance of NTP hydrolysis, great experimental effort has been devoted to study the hydrolysis mechanism for many years. These efforts include but are not limited to the structural determination of nucleotide-bound proteins through X-ray crystallography and cryo-electron microscopy,⁷ as well as the identification of essential residues through mutagenesis and

biochemical assays.⁸ Despite the success of such experiments, the specific molecular mechanism(s) of NTP hydrolysis often remains ambiguous because experiments cannot directly observe chemical reactions in proteins. As a result, theoretical studies, conducted through modeling and simulation, are highly desirable to reveal the hydrolysis mechanism at an atomistic level.

Even though molecular dynamics (MD) simulations have benefited enormously from the rise of computational capabilities over the last few decades,⁹ the accurate computational study of NTP hydrolysis remains very challenging for primarily two reasons. First, the nature of the hydrolysis reaction requires the chemical bonding to be modeled in a reactive manner (i.e., bonds will be broken and new ones will be formed). Quantum mechanics/molecular mechanics (QM/MM) simulation is one possible solution to this problem, in which the NTP molecules and nearby protein region

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undergoing and directly influencing the hydrolysis reaction are modeled with electronic structure theory, and the other parts of the system are modeled with classical molecular mechanics. However, the phosphate groups, catalytic metals, and their nearby amino acids and water molecules are all relevant to the hydrolysis reaction, usually making the reactive QM region larger than 150 atoms. Simulating a system of this size with electronic structure theory is extremely computationally expensive, even at the level of density functional theory (DFT).¹⁰ To overcome this challenge, researchers have developed various reactive models. Warshel and co-workers have proposed the empirical valence bond (EVB) approach,^{11,12} which uses a diabatic representation to describe the reactant, intermediate, and product states of the reaction and empirical interaction, as one way of studying reactions in complex systems. The EVB approach has been recently applied to the study of NTP hydrolysis.¹³ The multiscale reactive molecular dynamics (MS-RMD) approach,^{14–16} where the reactive potential energy surface is defined by an “on the fly” dynamically evolving linear combination of multiple diabatic potentials, and the terms of the interaction matrix defining them are variationally determined (at least in part) from QM/MM or *ab initio* MD forces, could in principle be applied to NTP reactions, as has been done to simulate proton transport in proteins (see, e.g., ref 17 for a recent example). The MS-RMD reactive potential energy surface enables the formation and cleavage of molecular bonds in a deterministic classical MD simulation. As another alternative, the self-consistent charge density functional tight binding (SCC-DFTB)¹⁸ might be utilized. This approach is a semiempirical QM algorithm that is derived from a Taylor expansion of the DFT Kohn–Sham energy for charge density fluctuations in which the Hamiltonian matrix elements are evaluated using a minimal basis set of pseudoatomic orbitals within a two-center approximation.^{19–21} SCC-DFTB has been applied to the ATP hydrolysis process in myosin.²² Another possible approach is to utilize force matching along a reaction pathway determined from less expensive semiempirical calculations such as SCC-DFTB and to then correct them to higher level QM calculations.²³ In general, all of the aforementioned methods are implemented in order to greatly increase the amount of statistical sampling in the simulation by making evaluations of the forces in the QM region of the hydrolysis reaction faster, ideally (but not commonly) with a systematic and small trade-off in accuracy.

The second challenge is that the free energy barrier of NTP hydrolysis is usually on the order of tens of kcal/mol, which makes conventional MD and QM/MM simulations of no practical use in calculating a potential of mean force (PMF) because the transition state region will barely be sampled during a feasible simulation time. In a recent QM/MM study of ATP hydrolysis in F1-ATPase,²⁴ Shigehiko et al. optimized the geometry of the hydrolysis compounds along the reaction path and constructed an approximate free energy surface with vibrational frequencies from QM calculations. This approach only provides free energy profiles for the stationary points (potential energy minima and transition states) instead of the entire reaction path, and, more importantly, the lack of accounting for configurational entropy from nonlinear fluctuations may cause significant errors in the free energy surface for enzymatic reactions.²⁵ Therefore, enhanced sampling methods have been employed in the studies of NTP hydrolysis to address this high energy barrier issue. For example, Yang et al.²² used umbrella sampling and the weighted histogram anal-

ysis^{26,27} in their SCC-DFTB/MM simulations to encourage the attack of lytic water to the gamma phosphate and the proton transfers between the lytic water and amino acid. More recently, metadynamics (MetaD)^{28–30} has been adopted in NTP hydrolysis simulations.^{31–33} MetaD accelerates the simulation by discouraging it from revisiting configurations that have already been sampled through constructing a history-dependent bias potential $V(s)$ to help the system to overcome large free energy barriers. An appealing feature of MetaD is its ability to explore reactions without an *a priori* guess of the detailed reaction path,³⁴ in contrast to umbrella sampling where one has to prepare the geometry for each window according to a hypothetical reaction path.

In spite of the past application of MetaD in assisting the PMF calculation of an NTP hydrolysis, the practical and robust convergence of an acceptably accurate and therefore computationally expensive QM/MM simulation with MetaD remains extremely difficult. Other than the sampling limitations of the QM/MM simulation discussed earlier, the complexity of NTP hydrolysis may demand more collective variables (CVs) than those solely associated breaking the bond between the beta phosphate and the gamma oxygen, e.g., the proton transfer between the lytic water and/or the amino acid through a water mediated “proton relay”.³¹ Additionally, different sets of CVs are sometimes required to properly sample the forward and backward reactions.³³ As a result, the quality of the convergence in these early QM/MM MetaD simulations could not be verified as rigorously as the theory suggests, e.g., by checking for the diffusive motion of the CVs and for agreement between multiple replicas.

It is noteworthy that these early QM/MM MetaD simulations have either utilized the original nontempered MetaD,²⁸ where the same Gaussian is added to the Hamiltonian along the simulation, or the well-tempered MetaD (WTMetaD),²⁹ where the Gaussian is scaled with respect to the current local bias potential. In nontempered MetaD, the ever-growing history dependent bias potential does not converge to the PMF modulo a constant but rather fluctuates around the desired final state.³⁰ A possible mitigation of this behavior is to take the time average of the bias potential after observing the diffusive motion of the system in the CV space. However, as pointed out earlier, the identification of diffusive motion can be subjective and itself is very difficult to achieve due to the limited sampling of NTP hydrolysis in expensive QM/MM simulation, thus making an extensive enough MetaD simulation to check for truly diffusive behavior is presently nearly impossible. WTMetaD overcomes the convergence issue³⁰ but presents an obvious trade-off between the exploration of the CV space in the early stages of the simulation and the asymptotic convergence rate.^{35,36} For example, WTMetaD that decreases the Gaussian height slowly builds up a fast-growing but noisy bias potential, hindering the convergence. In contrast, a fast decrease of the Gaussian heights generates a smooth bias potential but prevents it from escaping a minimum within a feasible QM/MM simulation time, especially when the barrier in the CV space is very large, which is usually the case in NTP (e.g., ATP) hydrolysis.

As demonstrated in our recent papers,^{35,36} the newly developed transition-tempered MetaD (TTMetaD)³⁵ has been shown to converge rapidly and asymptotically without sacrificing the exploration of the CV space in the early stages of simulations, in contrast to current other convergent MetaD methods. This aspect of TTMetaD obviously makes it very

appealing to study NTP hydrolysis in computationally expensive QM/MM simulations, as the sampling of the latter will always be limited due to the high cost of the QM force evaluations. Additionally, as pointed out by McGrath et al.,³² as well as in our own experience, in a longer MetaD simulation the bias potential may introduce irreversible changes in the system that dramatically delays the convergence or pushes the system into unstable regions and may even crash the QM/MM simulation. Therefore, it is also of interest to employ a self-limiting bias potential to avoid these issues and to properly focus the QM/MM free energy sample, by using a method such as our recently developed metabasin MetaD (MBMetaD).³⁷

The purpose of this paper is to demonstrate, through the specific example of ATP hydrolysis in actin, that a combination of TTMetaD and MBMetaD with QM/MM MD simulation can provide a systematic and rigorous estimate of the free energy pathway of NTP hydrolysis in proteins. We study here the ATP hydrolysis in monomeric and filamentous actin and compare the features of the two reactions, building on our earlier work³¹ with this more powerful and convergent combinations of simulation methods. ATP hydrolysis in actin plays an important role in the actin-based cytoskeleton.^{38,39} For example, ATP-bound monomeric globular actin (G-actin) is added to the (+) end of an actin filament, undergoing conformational change to a more flattened state in filamentous actin (F-actin), which then catalyzes ATP hydrolysis, then releases the hydrolyzed phosphate group, and finally dissociates at the (−) end, now as an ADP-bound G-actin. The high selectivity for hydrolyzing ATP in F-actin, as opposed to in G-actin, is important for modulating the filament's physical properties and the binding affinity of various actin-binding proteins. The most recent experimental studies^{2,40} have reported the ATP hydrolysis rate to be $0.3 \pm 0.1 \text{ s}^{-1}$ and $7 \times 10^{-6} \text{ s}^{-1}$ for the reaction in F-actin and G-actin, respectively. Assuming the same Arrhenius prefactor in both forms of actin, this dramatic speedup suggests a $6.6 \pm 0.7 \text{ kcal/mol}$ hydrolysis free energy barrier difference between F- and G-actin. McCullagh et al.³¹ carried out a QM/MM MD computational study to reveal the origins of the different rates of ATP hydrolysis in the different actin states. Nontempered MetaD was employed in that work, and the simulations showed an 8 kcal/mol hydrolysis barrier difference between F- and G-actin, in good agreement with the experimental estimate. The water structure of the "proton relay" between the lytic water in the hydrolysis site and Asp154 was found to be to one difference between F-actin and G-actin.

In this paper, we revisit these same actin systems and compare the performance of TTMetaD simulation against WTMetaD and nontempered MetaD simulation. Our simulation follows the setup of McCullagh et al.,³¹ except for using different MetaD approaches. In particular, we show that reaching convergence requires a comprehensive representation of the overall hydrolysis process by also adding in an additional CV that biases proton transfer from the nucleophilic water molecule. This additional concurrent metadynamics benefits from the self-limiting feature of MBMetaD and dramatically accelerates the convergence of the free energy surface. Most importantly, our simulations provide a rigorous analysis of the convergence of the bias potential, which has so far largely been overlooked in QM/MM simulations of NTP hydrolysis. The focus and conclusions of this paper reveal the computational advantages of utilizing TTMetaD and MBMetaD with QM/MM MD when studying NTP hydrolysis in proteins. Future

work will focus on utilizing this approach to then study the hydrolysis mechanism itself in key proteins.

METHODOLOGY

Transition-Tempered Metadynamics. MetaD utilizes an external history dependent bias potential, which has the form of a summation of Gaussian functions centered at previously visited points in a preselected CV space. The bias potential helps the chemical system to overcome large free energy barriers by discouraging it from revisiting configurations that have already been sampled. In the first implementation of MetaD, the bias potential (V_G) evolved according to the equation

$$V_G(s, t) = \int_0^t dt' w \exp\left[-\sum_{i=1}^d f(S_i(R))\right] \quad (1)$$

with w called the bias energy addition rate, usually expressed as the height of individual Gaussian functions added, w_0 , and divided by a deposition stride τ . The function $S_i(R)$ represents the i^{th} CV for MetaD simulations, i.e., distance, angle, dihedral, atomic coordinates, radius of gyration, local density, etc. The term $f(S_i(R))$ is defined as

$$f(S_i(R)) = \frac{(S_i(R) - S_i(R(t')))^2}{2\sigma_i^2} \quad (2)$$

in which σ_i is the width of the Gaussian for the i^{th} CV. This approach is usually referred to as nontempered MetaD because the bias energy addition rate w does not depend on the progress of the simulation. The bias potential from nontempered MetaD does not converge asymptotically,³⁰ and a practical solution is to take the time average of the bias potential, after the time (t_0) when the motion of the system on the CV space becomes diffusive.⁴¹ The PMF estimated from nontempered MetaD takes the form

$$\bar{V}_G(t) = \frac{\int_{t_0}^t V_G(t) dt}{\int_{t_0}^t dt} \quad (3)$$

where $V_G(t)$ is the instantaneous bias potential.

To address the convergence issue, WTMetaD tempers the Gaussian heights exponentially with respect to the local bias potential. The height of the Gaussian function becomes time-dependent

$$w(t) = w_0 \exp\left[-\frac{V_G(s, t)}{k_B \Delta T}\right] \quad (4)$$

in which ΔT is a parameter (with the unit of temperature) chosen prior to the simulation. Dama et al. have provided a rigorous mathematical proof that WTMetaD converges asymptotically to a linearly scaled inverse of the underlying PMF.³⁰

On the other hand, TTMetaD smoothly converges the bias potential asymptotically like WTMetaD, but it keeps track of the region that the bias potential has explored and delays the tempering of the Gaussian height until all the basin points are included in this region. Because the basins are relatively filled prior to the tempering, TTMetaD can be used with a quickly converging tempering (small ΔT) without a concern for being trapped in the first nearby free energy minimum. The height of the Gaussian in TTMetaD is tempered as

$$w(t) = w_0 \exp\left[-\frac{V^*(s(\lambda), t)}{k_B \Delta T}\right] \quad (5)$$

where $V^*(s, t)$ is the minimal bias on the maximally biased path among all the continuous paths $s(\lambda)$ that connects all of a set of preselected basin points in the CV space. TTMetaD does not require the preknowledge of the barrier height to tune ΔT . Instead, one needs to estimate the position of the basin points in the CV space. This estimation is straightforward when it is defined by the problem of interest, i.e., in this case the ATP state and ADP + Pi state for the ATP hydrolysis reaction. Generally, TTMetaD has been proven to be robust to the accuracy of the basin points as long as they are separated by a major barrier.³⁶

Metabasin Metadynamics. In a conventional MetaD simulation, the bias energy is constructed to flatten the underlying free energy surface globally on the CV space. However, in many-dimensional systems, the system may be pushed out from the low energy regions that are of interest to some of the higher energy regions that are not physical or as relevant. Worse, entering these high-energy regions can couple with large, effectively irreversible conformation changes, introducing large hysteresis and limiting reproducibility in the estimates of the PMF. Therefore, it is highly desirable to use a self-limiting biasing method that only fills the underlying free energy surface up to a specified level and then converges that bias potential to estimate the PMF. This forces a more physically relevant exploration and greater reproducibility of the PMF estimates.

MBMetaD achieves this self-limiting behavior for the bias potential by only flattening a finite domain (D) of the whole CV space. MBMetaD therefore utilizes a Gaussian with two parts

$$f_{\text{MB}}(S_i(R)) = f_{\text{Int}}(S_i(R)) + f_{\text{Ext}}(S_i(R)) \quad (6)$$

in which $f_{\text{Int}}(S_i(R))$ is the part to flatten the interior of the domain, and $f_{\text{Ext}}(S_i(R))$ raises the bias level of the exterior to match the bias level of the domain boundary to counteract the force from $f_{\text{Int}}(S_i(R))$ that slopes into the exterior of the domain. The term $f_{\text{Int}}(S_i(R))$ is rescaled from the regular Gaussian (named $f_0(S_i(R))$) following a relative to the multiplicative McGovern-de Pablo rule⁴²

$$f_{\text{Int}}(S_i(R)) = f_0(S_i(R))/f(I(s; D, f_0)) \quad (7)$$

with I being the boundary-normalized integral of $f_0(S_i(R))$ over D , and f is a scaling function. The function $f(x)$ evaluates as x for $x \geq 1$ and $x + 0.5*(1-x)^2$ for $x < 1$, so that these hills are identical to the McGovern-de Pablo form inside the domain but taper more smoothly to zero outside of the domain. At the same time, MBMetaD constructs the exterior bias update, $f_{\text{Ext}}(S_i(R))$, as the function that would force the sampling into the domain near the boundary exactly enough to cancel out how the interior bias, $f_{\text{Int}}(S_i(R))$, would push the sampling out near the domain boundary if sampling inside the domain were perfectly flat (or were the desired tempered distribution in the case of WTMetaD). A detailed description of MBMetaD and additional applications can be found in ref 37.

Initial Structures. The structures of both G- and F-actin were generated from equilibrated classical molecular dynamics simulations.⁴³ The crystal structure (pdb code 1NWK) was used for the G-actin simulation and the Oda model (pdb code 2ZWH) after equilibration of a 13-mer periodic filament was

used for F-actin simulation. The main change during the actin polymerization is a flattening of the subdomain 2–1–3–4 dihedral, which is -0.55 degree in F-actin and -27.09 degree in G-actin. To prevent F-actin from relaxing to G-actin during the MetaD simulation, a harmonic bias force with a spring constant of 228.8 kcal/mol was applied to restrain this dihedral based on a coarse-grained mapping of F-actin. Further details of the structures in this simulation are provided in McCullagh et al.³¹ and its Supporting Information.

QM/MM Setup. We adopted the same QM region as in McCullagh et al.,³¹ including methyl triphosphate, bound magnesium, surrounding waters, and ten amino acids (Asp11, Gly13, Ser14, Gly15, Lys18, Gln137, Asp154, Gly156, Asp157, and His161). The atoms involved in coordination with the catalytic magnesium, i.e., one beta oxygen, one gamma oxygen, and four water molecules, are included in this QM region. Sequential amino acids backbone atoms were included and hydrogen capped at the terminal nitrogen and carbon. Nonsequential amino acids were truncated at the C_β or C_γ carbon and capped with hydrogens.³¹ The PBE/TZV2P level of density functional theory^{44,45} was employed for the QM region simulations, and the magnesium ion was treated with GTH pseudopotentials.^{46,47} The CHARMM27 force field^{48,49} was used to model the MM region, and periodic boundary conditions were applied in the NVT ensemble with a smoothed particle mesh Ewald treatment of the long-range electrostatics.⁵⁰ The temperature was maintained at 310 K with a Nosé–Hoover thermostat,⁵¹ and the integration time step was set to 0.5 fs, totaling over 1.5 ns of simulation time. The total CPU time used in this project was about 5 million hours. The QM/MM MetaD simulations were performed with the CP2K software⁵² coupled to the enhanced sampling package PLUMED2.⁵³

Metadynamics Setup. A set of appropriate CVs (denoted as “ s ”) representing the chemical reaction of interest must be chosen prior to MetaD simulation. As described earlier, MetaD sequentially builds up a history dependent bias potential of these variables to accelerate their dynamics. As suggested by McCullagh et al.,³¹ our TTMetaD and WTMetaD simulations utilize two CVs in the ATP hydrolysis reaction (cf. Figure 1). One CV is the coordination number between the terminal (gamma) phosphorus (P_γ) and bridging (beta) oxygen (O_β), shown in Figure 1. This coordination number describes the forming and cleavage of the bond between P_γ and O_β and allows the recombination of P_γ with any of the O_β atoms. The

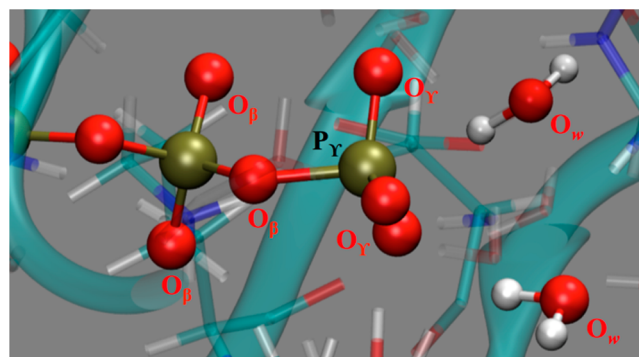


Figure 1. An illustration of the atoms involved in the two collective variables employed in the TTMetaD simulations of ATP hydrolysis in actin.

other CV, also seen from Figure 1, is the coordination number between P_γ and both gamma oxygen (O_γ) and QM water oxygens (O_w). Therefore, all the QM water molecules (about 30) can possibly be the lytic water that attacks P_γ and triggers the hydrolysis reaction, in contrast to only biasing a particular water molecule in the QM region to be the lytic water.³³ We anticipate this species-agnostic approach to be important to prevent unphysical artifacts from breaking chemical symmetries while modeling this reaction.

It should be noted that the CVs in our simulation prefer neither an “associative” mechanism^{54,55} nor a “dissociative” mechanism^{56,57} for the ATP hydrolysis. The former suggests the lytic water molecule forms a bond with P_γ before the bond between P_γ and O_β breaks. The latter proposes the P_γ and O_β bond breaks before the nucleophilic attack of lytic water. Importantly, an additional CV that biases the hydrogen transfer from the lytic water was also used in our QM/MM TTMetaD simulation with a self-limiting MetaD (MBMetaD) to enhance convergence of the PMF. This feature of the simulation will be further described later.

The parameter ΔT (in eq 4) that tunes the speed of decreasing Gaussian height significantly affects WTMetaD convergence rate.^{29,35,36,58} It is suggested in the original WTMetaD paper that ΔT should be chosen according to the free energy barrier, as $\Delta T + T$ should be of the order of magnitude of the barrier height.²⁹ Therefore, ΔT was chosen to be 12090 K (corresponding to 24.0 kcal/mol) to maximize the efficiency of WTMetaD, based on the previously determined hydrolysis barrier height (30 and 22 kcal/mol for ATP hydrolysis in G- and F-actin, respectively).³¹ However, it is important to note that this level of accuracy of the estimates of the energy barrier is almost impossible for every NPT hydrolysis reaction, especially if one is interested in a reaction that has not been studied before. We note that TTMetaD is more robust to the choice of ΔT as compared to WTMetaD,^{35,36} and it is recommended to temper more aggressively (with a smaller ΔT value), since the tempering is not applied until the basins are already relatively filled. We therefore set ΔT to be 1240 K (corresponding to 2.5 kcal/mol) for the TTMetaD simulations. Additionally, one needs to provide the estimates of the basin positions, which are easy to do for ATP hydrolysis (one basin is the ATP region and the other is the ADP + Pi region). The same heights of Gaussian are used for both WTMetaD and TTMetaD simulations as in the previous nontempered MetaD simulations.³¹

RESULTS

TTMetaD vs WTMetaD. We carried out three sets of QM/MM simulations to compare the performance between WTMetaD and TTMetaD in the early stages of the simulation: F-actin starting from ATP, F-actin starting from ADP + Pi, and G-actin starting from ATP. For hydrolysis in F-actin, WTMetaD and TTMetaD simulations were initiated separately from ATP and ADP basins. To illustrate the data, the bias potentials from the ATP and ADP replicas were summed and plotted together into one plot (top row in Figure 2), following the practice of multiple walker MetaD.⁵⁹ This figure demonstrates the efficiency of TTMetaD in exploring the CV space at the early stages of the simulation. It is seen that after 50 ps, both ATP and ADP + Pi basins of the underlying free energy surface are much better filled in TTMetaD simulations than in WTMetaD simulations. In the TTMetaD simulations, both ATP and ADP replicas have escaped from their origin

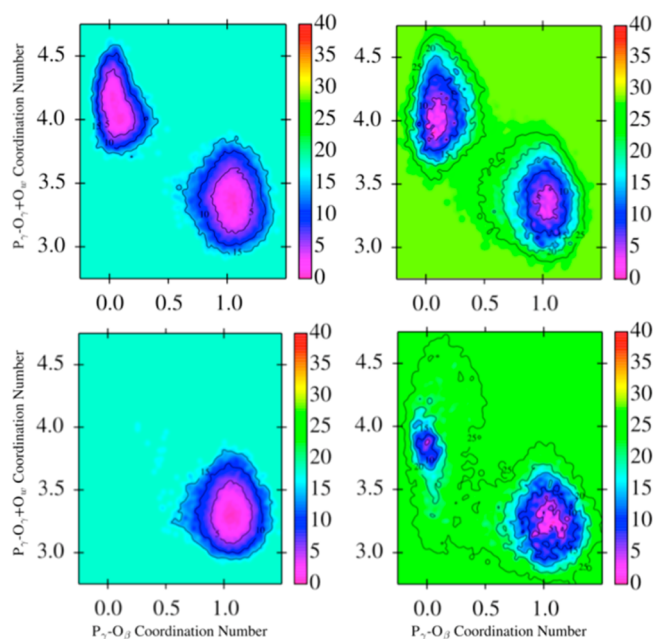


Figure 2. Inverse of the bias potential for ATP hydrolysis in F-actin (top row) and G-actin (bottom row). For F-actin, the bias potential is the summation of ATP and ADP+Pi replicas (50 ps each). For G-actin, the bias potential is from one of two ATP replicas (50 ps). Bias potentials from WTMetaD are shown in the left column, and bias potentials from TTMetaD are shown in the right column. The unit of energy is kcal/mol.

basin and started to explore the transition state region, in contrast to them being trapped in its origin basin in WTMetaD simulations. The WTMetaD contour lines are much smoother than those in the TTMetaD plot, reflecting the fact that the WTMetaD is using smaller Gaussians before overcoming the barrier. The maximum value of the bias energy, which can be identified from the background color in each figure, is about 25 kcal/mol in TTMetaD, significantly higher than the value of 18 kcal/mol in WTMetaD. According to the tempering rules of WTMetaD (eq 4), a continued WTMetaD simulation would use as small as 47% of the original Gaussian hill height, in contrast to TTMetaD that still uses a full Gaussian since the predefined basins have not been connected by the bias potential yet. WTMetaD will eventually converge with longer simulation times; however, due to tempering, it will take even longer to add the same amount of bias energy to the system than before, and this makes it relatively inefficient. The G-actin ATP simulations show a very similar story to the F-actin simulations (bottom row in Figure 2).

At this point, the comparison between TTMetaD and WTMetaD was clear, so we halted the WTMetaD simulations at 50 ps. It is important to emphasize that WTMetaD was set up in a way to maximize its efficiency by taking advantage of an accurate prior knowledge of the hydrolysis barrier height,³¹ but TTMetaD’s advantages seen early in the simulation were compelling. Indeed, the early exploration efficiency of TTMetaD appears to be of considerable value to the computationally demanding QM/MM NTP hydrolysis simulations.

Practical Convergence Requires Comprehensive CVs.

We next continued all three sets of TTMetaD simulations (F-actin starting from ATP, F-actin starting from ADP + Pi, and G-actin starting from ATP) beyond 50 ps to achieve better PMF

convergence. We also replicated each set, so that the hydrolysis in G-actin and in F-actin was both modeled by 4 replicas each. After reading in the TTMetaD bias potential as shown in Figure 2, the replicated trajectories were restarted from the configuration at 50 ps with a random velocity sampled from a Boltzmann distribution at 310 K. The reported PMF estimates are then averaged over those replicas, because this ensemble-average approach has previously proven to converge the MetaD simulations faster in both wall times and CPU times.³⁶

As the TTMetaD simulation goes on, the trajectories show diffusive motion between ATP and ADP + $[\text{H}_2\text{O}-\text{PO}_3]^-$. However, a complete hydrolysis reaction requires one of the protons from the lytic water in $[\text{H}_2\text{O}-\text{PO}_3]^-$ to transfer to O_γ to form $[\text{H}_2\text{PO}_4]^-$ (see Figure 3);^{24,31,33} but, without an

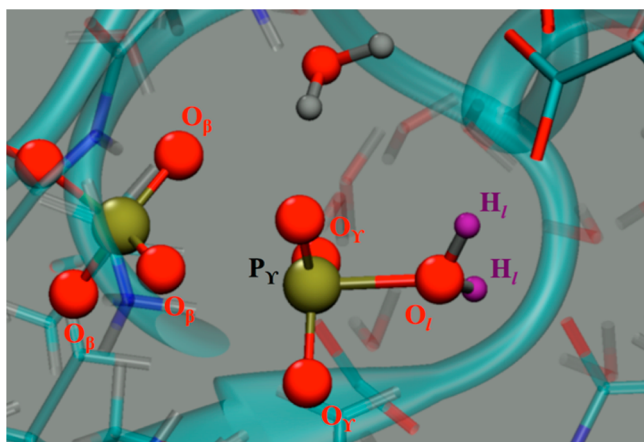


Figure 3. An illustration of the collective variables employed in the MBMetaD simulations to accelerate the transition between $[\text{H}_2\text{O}-\text{PO}_3]^-$ (bound water proton coordination number is 2, shown as purple) and $[\text{H}_2\text{PO}_4]^-$ (bound water proton coordination number is 1, not shown).

additional bias, $[\text{H}_2\text{O}-\text{PO}_3]^-$ (seen in Figure 3) remains a metastable state, and the transition between $[\text{H}_2\text{O}-\text{PO}_3]^-$ and $[\text{H}_2\text{PO}_4]^-$ does not happen spontaneously.³³ The $[\text{H}_2\text{O}-\text{PO}_3]^-$ and $[\text{H}_2\text{PO}_4]^-$ correspond to a very similar value on the 2D CV space (with only slight differences due to surrounding water structure), and, as a result, the 2-CV biasing force from the previously described TTMetaD simulations did not accelerate this proton transfer process. The neglect of an important slow degree of freedom (i.e., rare event) in MetaD simulations usually leads to significant hysteresis in the PMF estimates,⁶⁰ and we observed this in our simulation. Taking the ATP hydrolysis in G-actin as an illustrative example, the longer simulation shows the trajectories spend a relatively longer time between the $[\text{H}_2\text{O}-\text{PO}_3]^-$ and the transition state region, leading to an unphysical minimum near that region in our PMF estimates (right panel in Figure 4).

In general, MetaD simulations with more than 2 instantaneous CVs are difficult to converge even with tempering.^{37,60,61} One solution to this higher-dimensional problem is to simulate a series of MetaD simulations with different concurrent CVs and let their bias energy and configuration exchange between one another.⁶² However, the application of this approach to the ATP hydrolysis system seems less than optimal, as the number of replicas needed to build “the ladder of replicas” could be large and QM/MM simulation is costly.

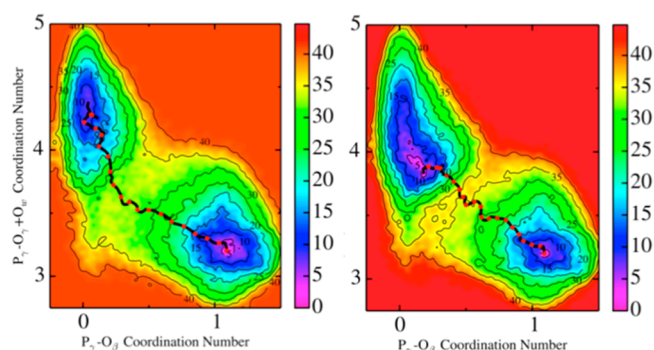


Figure 4. Bias potential from simulations of ATP hydrolysis in G-actin. Left panel: the inverse of the bias potential from averaging over 4 TTMetaD replicas with 2 CVs (180 ps for each replica). Right panel: the extended (~50 ps) simulation from the left panel. An unphysical hysteresis builds up in the PMF (~0.1, ~3.8) because of the neglect of a third key slow degree of freedom (proton transfer of the lytic water). The unit of energy is kcal/mol.

Nevertheless, we decided to explore the idea of concurrent MetaD⁶² and use it to explore just a third CV to accelerate the hydrogen transfer of the lytic water: the coordination number between lytic water oxygen (O_l) and lytic water hydrogen (H_l). This CV was chosen to bias the transition between $[\text{H}_2\text{O}-\text{PO}_3]^-$ (proton coordination number of the bound water is 2) and $[\text{H}_2\text{PO}_4]^-$ (proton coordination number of the bound water is 1). An illustration of this CV can be found in Figure 3. As shown in other research, as well as in our own simulations, the lytic water, instead of an already dissociated hydroxide,^{32,33} nucleophilically attacks the backside of P_γ and initiates the hydrolysis reactions. Therefore, biasing the lytic water from the beginning of the simulation has a risk of introducing artifacts into the simulation, i.e., forcing hydroxide to be the attacking group, if the MetaD drives autoionization too aggressively. Additionally, the lytic water could be any water molecule adjacent to the P_γ , and biasing all of them to the point of autoionization is clearly undesirable.

Therefore, we utilized MBMetaD to introduce a concurrent and self-limiting bias potential that features the following properties: 1) it is only in play after the formation of the $[\text{H}_2\text{O}-\text{PO}_3]^-$ intermediate; 2) it applies only to the lytic water that forms the $[\text{H}_2\text{O}-\text{PO}_3]^-$ intermediate; and 3) the maximum bias value applied to the CV is less than 6 kcal/mol (self-limiting). The value of 6 kcal/mol was chosen as an upper-limit so that the O_l-H_l bond would not break unless there is a good H_l accepting group close by, in contrast to conventional MetaD where the O_l-H_l bond would be driven to break regardless of the local environment. As a result of this MBMetaD bias, the weakened O_l-H_l bond had a much shorter lifetime than in the 2-CV TTMetaD simulations, and the transfer between $[\text{H}_2\text{O}-\text{PO}_3]^-$ and $[\text{H}_2\text{PO}_4]^-$ became diffusive. Then, depending on the chemical environment, the proton transfers from lytic water oxygen to gamma oxygen followed different pathways.

TTMetaD combined with concurrent MBMetaD is able to converge the simulation well, and the final PMF estimates from averaging over four replicas are depicted in Figure 5. The minimum free energy path was then computed with the string method⁶³ and is plotted in Figure 6. The difference in activation free energy of ATP hydrolysis between G-actin and F-actin is 7.1 kcal/mol, aligning well with the experimentally suggested difference of 6.6 ± 0.7 kcal/mol. This agreement is

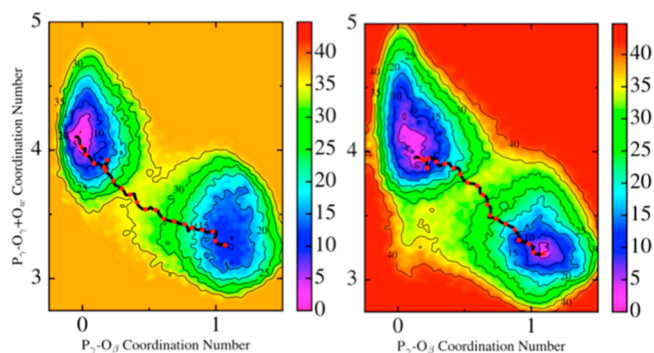


Figure 5. ATP hydrolysis in F- (left) and G-actin (right) PMF from TTMetaD + concurrent MBMetaD simulations as described in the main text (averaged over 4 replicas). The unit of energy is kcal/mol.

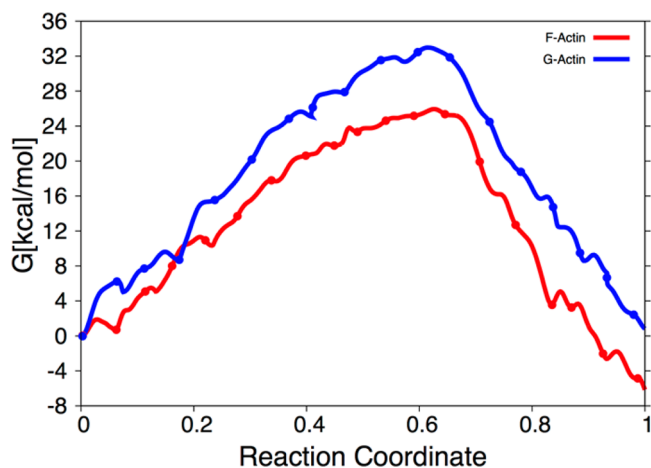


Figure 6. Minimum free energy path from the 2D free energy surfaces (Figure 5) for ATP hydrolysis in F- and G-actin. The reaction coordinate represents the hydrolysis reaction progress along the minimum free energy path, i.e. “0” being ATP and “1” being ADP + Pi.

also closer than the previous result of 8 kcal/mol obtained from nontempered MetaD simulations.³¹ The overall magnitude of the hydrolysis barrier is almost certainly dominated by electrostatic effects in the active site as well as the energy required to break the chemical bonds plus differences in the efficiency of the proton relay from the lytic water in F-actin versus G-actin.³¹

Discussions of Convergence. The asymptotic convergence for tempered metadynamics (WTMetaD and TTMetaD) cannot in principle be reached with finite sampling.³⁰ Therefore, it is important to set up practical convergence criteria that can be verified for expensive QM/MM simulations such as the present ones of ATP hydrolysis. As suggested in a number of papers,^{28,36,60,64} the diffusive motions of the CVs and the differences between the replicas can be employed as convergence criteria in MetaD simulations. Conversely, the evolution of the bias potential is not used because of a concern for false convergence, where the Gaussian height could have been tempered so little that the bias potential barely changes with longer simulation, but the calculation is in fact not converged.

To verify the diffusive motion of the system in CV space, we restarted trajectories from the ATP configuration with velocities randomly sampled from a Boltzmann distribution (at 310 K). The “candidate” PMF (Figure 5) was read in, and the Gaussians kept being added following the TTMetaD tempering rule. The concurrent and self-limiting bias accelerating the transfer between $[\text{H}_2\text{O}-\text{PO}_3]^-$ and $[\text{H}_2\text{PO}_4]^-$ was applied as described before. If the previous simulation had indeed reached a reasonable degree of convergence, one would expect the system to escape from the ATP basin, cross the transition state region, form $\text{ADP} + [\text{H}_2\text{O}-\text{PO}_3]^-$, and eventually reach $\text{ADP} + [\text{H}_2\text{PO}_4]^-$ relatively quickly, i.e., the whole process should be diffusive without being trapped in any region. Taking F-actin as an example, we tested eight such trajectories, and all of them show satisfactory diffusive motion (i.e., by finishing the hydrolysis process within 15 ps, which includes constructing the self-limiting bias for the hydrogen transfer CV). An example

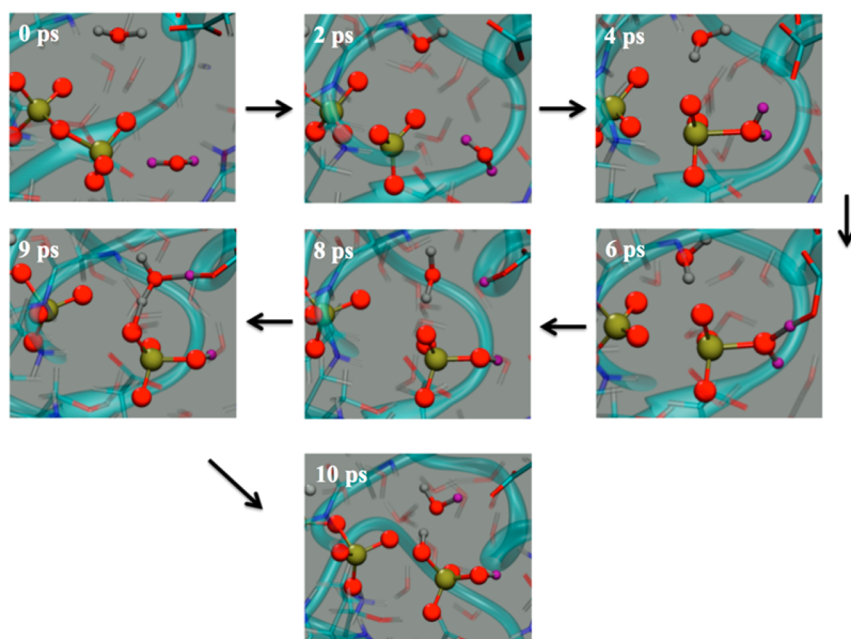


Figure 7. An example of a diffusive trajectory of ATP hydrolysis in F-actin.

trajectory of ATP hydrolysis in F-actin that demonstrates the proton transfer is shown in Figure 7. The evolution of the CVs for another testing trajectory of hydrolysis in F-actin is depicted in the Supporting Information (Figure S1).

We also compared the differences between the bias potentials from each replica because the robust convergence cannot be confirmed until they agree reasonably well with every other. As mentioned earlier, we simulated four replicated simulations for ATP hydrolysis in both F- and G-actin. Figure 8 depicts the

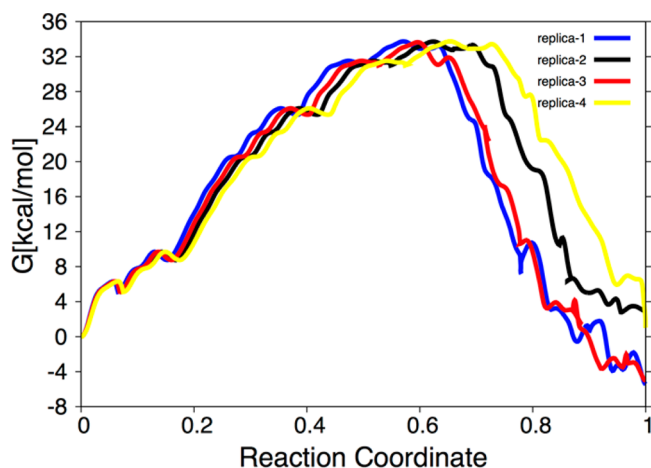


Figure 8. Minimum free energy path from 4 replicas of ATP hydrolysis in G-actin. The reaction coordinate represents the hydrolysis reaction progress along the minimum free energy path, i.e. “0” being ATP and “1” being ADP + Pi.

minimum free energy paths (the ATP region is shifted to zero) from the four replicas of ATP hydrolysis in G-actin. All four minimum free energy paths show good agreements in the hydrolysis barrier. However, differences in the ADP + Pi region indicate that the convergence quality is not as ideal as in the ATP transition state region. Nevertheless, in actin the forward ATP hydrolysis reaction (forming ADP + Pi) is much more important than its synthesis (reverse reaction), which is a very rare event.

It is also of interest to compare the converged TTMetaD and concurrent MBMetaD simulation with the previous nontempered MetaD simulation from McCullagh et al.³¹ The PMF estimates and the minimum free energy path from the nontempered simulation are replotted in the Supporting Information (Figures S2 and S3). Even though there are differences in the PMF estimates between the two studies, the key feature, the barrier height in the minimum free energy paths from both simulations, agrees well. As has been discussed in the Introduction, a QM/MM study of NTP hydrolysis is extremely expensive and complicated; therefore, McCullagh et al.³¹ had chosen to employ nontempered MetaD to ensure the exploration of the CV space rather than to aim for a more meaningful PMF convergence with a method like WTMetaD. Also, there was a lack of averaging of the nontempered MetaD bias after observing the diffusive motion; hence the convergence of the nontempered MetaD PMF estimate was not ideal. However, as shown here with the recently developed TTMetaD and concurrent MBMetaD, QM/MM simulations are now able to provide a rigorous and converged PMF estimate for this system and presumably others similar to it.

CONCLUSIONS

Protein mediated NTP hydrolysis such as the actin ATP hydrolysis studied in this work remains one of the most important and challenging biochemical reactions in computational biophysics. The size and complexity of the protein environment adjacent to the hydrolysis site (water structure, catalytic metal, and amino acid residues), as well as the chemical complexity of the hydrolysis reaction itself, limits the amount of sampling that one can achieve in a computationally demanding QM/MM simulation. Therefore, an enhanced sampling method featuring high-quality PMF estimates at the earliest possible stage of the simulation is highly desirable. As shown in this work, TTMetaD is well suited to this task in that it explores the CV space with full Gaussians until the basins are roughly filled. This gives rapid estimates containing all of the key features of the PMF. The bias is then aggressively converged using quickly tempered Gaussians for the asymptotical convergence. The TTMetaD QM/MM simulations also exhibit diffusive motion between ATP and ADP + [H₂O⁻PO₃]⁻.

We further introduced an additional bias potential to accelerate the transition between [H₂O–PO₃]⁻ and [H₂PO₄]⁻. To perturb the hydrolysis dynamics to the least effective extent, this bias was constructed from the self-limiting MBMetaD method. It is important to emphasize that our MetaD simulations were not carried out with 3 simultaneous CVs, where one must compute the PMF with respect to any two CVs at the fixed value of the third CV. The third CV (biasing proton transfer from lytic water) was only introduced to accelerate one slow degree of freedom that prevented the TTMetaD with other two hydrolysis CVs from converging. We therefore consider TTMetaD + concurrent MBMetaD to be a much more efficient and physically motivated approach than MetaD simulations with 3 simultaneous CVs. The reasons are 1) The larger number of simultaneous CVs that MetaD uses, the longer the simulation takes to converge; 2) Biasing the lytic water molecules at the outset of the simulation can lead to cleaving the O–H bond before it nucleophilically attacks ATP; and 3) Identifying in advance a particular water molecule to be the lytic water from all of the QM water molecules is nontrivial, so it is optimal to allow one to initiate the reaction before adding the MBMetaD bias to it.

We have also shown that running replicated tempered MetaD simulations is advantageous, since not only do they provide confirmation of convergence, but they can also reduce the simulation wall clock times, thus allowing them to converge faster. Furthermore, a single tempered MetaD calculation with limited sampling can be very untrustworthy.³⁶ In principal, nontempered MetaD can also provide converged PMF estimates with two levels of averaging: time averaging over a period of the simulation after it becomes diffusive and replica averaging over the time-averaged bias potential. However, the diffusive motion itself is difficult to realize due to the limited sampling in NTP hydrolysis. To the best of our knowledge, this work is the first time that diffusive MetaD behavior has been demonstrated for a QM/MM simulation of an NTP hydrolysis reaction.

The enhanced sampling methods described in this paper, TTMetaD and MBMetaD, have been implemented in a publicly available fork of the latest version of PLUMED2 and are free to use. We are currently working on implementing TTMetaD and MBMetaD into the public version of the most

recent PLUMED. Both TTMetaD and MBMetaD are easy to understand and apply, and, compared to a conventional nontempered MetaD or WTMetaD simulation, the extra input parameters that TTMetaD and MBMetaD require have robust defaults and are straightforward to incorporate. A detailed user manual for these two methods is available upon request. No specialized or separate mathematical libraries are required by the new features of these methods, thus any researcher already using MetaD and PLUMED2 can begin using TTMetaD and MBMetaD simply by reinstalling the new fork of PLUMED2 with any compatible MD engine. An example MBMetaD input file (used in our simulation) is provided in the [Supporting Information](#).

To conclude, in this paper we have demonstrated the combined advantages of TTMetaD and MBMetaD simulation for demanding QM/MM MD free energy simulations of the ATP hydrolysis process in actin. In turn, the calculated (and demonstrably reasonably well converged) difference in free energy barriers for G-actin versus F-actin is found to be in good agreement with the experimental value. Our work therefore serves as an example for utilizing this approach in simulating complex NTP hydrolysis reactions in proteins, which are some of the most challenging of all biochemical reactions. Additional simulations along these lines will be the target of future research, as well as additional mechanistic analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jctc.7b00077](https://doi.org/10.1021/acs.jctc.7b00077).

Evolution of the CVs for the ATP hydrolysis in F-actin, Figure S1; PMF estimates and the minimum free energy path from the nontempered simulation, Figures S2 and S3, respectively; example MBMetaD input file ([PDF](#))

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Notes

The authors declare no competing financial interest.

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