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Domain motion of individual F_1 -ATPase β -subunits during unbiased molecular dynamics simulations

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Abstract

F_1 -ATPase is the catalytic domain of F_1F_0 -ATP synthase and consists of an hexameric arrangement of three non-catalytic α and three catalytic β subunits. We have used unbiased molecular dynamics simulations with a total simulation time of 900 ns to investigate the dynamic relaxation properties of isolated β -subunits as a step toward explaining the function of the integral F_1 unit. To this end, we simulated the open (β_E) and the closed (β_{TP}) conformations under unbiased conditions for up to 120 ns each using several samples. The simulations confirm that nucleotide-free β_E retains its open configuration over the course of the simulations. The same is true when the neighbouring α subunits are included. The nucleotide-depleted as well as the nucleotide-bound isolated β_{TP} subunits show a significant trend toward the open conformation during our simulations, with one trajectory per case opening completely. Hence, our simulations suggest that the equilibrium conformation of a nucleotide-free β -subunit is the open conformation and that the transition from the closed to the open conformation can occur on a timescale of a few tens of nanoseconds.

Introduction

In living organisms, most of the energy derived from light and food has to be transformed in an efficient manner into adenosine triphosphate (ATP), the universal energy carrier. The enzyme responsible for this task in bacteria, chloroplasts and mitochondria is ATP synthase, a large protein complex of two coupled rotary molecular motors, F_1 and F_0 . F_0 is membrane embedded and driven by a transmembrane proton gradient. The present work focuses on the F_1 unit which contains the catalytic sites responsible for ATP generation and converts mechanical energy into chemical (bond) energy.

The first crystallographic structure of F_1 -ATPase was resolved in 1994 for bovine mitochondrial ATPase¹ and revealed that F_1 consisted of three α and three β -subunits in a hexameric arrangement. The catalytic sites themselves were located at the interface of adjacent α and β units and were named according to the nucleotide occupancy in the original F_1 structure:¹ β_{TP} for bound ATP, β_{DP} for bound ADP and β_E for empty. From biochemical equilibrium binding studies we know that all three β -subunits have different binding affinities for ATP at any given time. Unfortunately, and despite much research, it is still not

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clear how to map the observed structural states of the β -subunits to their measured biochemical affinities for ATP. The closed β -subunit β_{TP} unit was often assumed to have the highest ATP affinity¹ but later studies argued that the β_{DP} site has the highest ATP affinity.² Recently it was found experimentally³ that the β_{TP} site is indeed the high-affinity site in agreement with the studies by Yang et al.⁴

Because of the importance of F_1 -ATPase a significant number of computational studies have been performed to investigate its function. Some of these studies investigated the structure-function relationship mainly based on structural information from X-ray studies and biochemical experiments.^{2,5–8} Oster et al. have performed studies based on structural interpolation,^{9–11} some combined with molecular dynamics (MD) simulations.^{12,13} In one of these studies¹³ it was concluded that a β -sheet within the β -subunit acts as a spring that stores a considerable amount of elastic energy. Normal mode analyses further explored the inherent flexibility of the α and β -subunits.^{14,15} The flexibility of the subunits and the whole complex was also the topic of an investigation using dynamical domain analysis.¹⁶ Structure-based models have been proposed treating both the synthesis and hydrolysis mode of F_1 operation¹⁷ and detailed simulations of the binding pockets and of the nucleotide binding energetics have been performed.^{4,18–21} By now, several fully atomistic MD simulations of the complete F_1 complex have been performed.^{22–25} In addition, functional rotations, e.g., in the efflux pump AcrB, have been studied using targeted MD simulations.²⁶ Unfortunately, due to its size MD simulations of the whole F_1 -complex are computationally demanding and can only be performed for relatively short time-scales (compared to the timescale of the catalytic event) or require the application of external forces.

These shortcomings suggest the simulation of isolated subunits as a means to investigate the modus operandi of the full F_1 complex. Of particular interest are the β subunits since they house the catalytic sites and are thought to undergo large conformation changes during the hydrolysis cycle. In an earlier study²⁷ 12-ns long unbiased molecular dynamics simulations (also known as “free dynamics” simulations) were performed while in a recent investigation by Ito and Ikeguchi²⁸ the time lengths of the free dynamics was extended to 100 ns. The latter study shows that the average conformation of β_E remains open and does not show spontaneous closing of isolated nucleotide-free β -subunits on the simulation time scale in contrast to what was reported earlier.²⁷ These findings are in agreement with NMR studies by Yagi et al.²⁹ who concluded that the β -subunits take an open form in the absence of bound nucleotide and are also in agreement with the simulation studies reported in Ref. 13. Recently, the NMR studies were extended to include mutations of the β -subunits to determine the residues involved in the ADP/ATP-binding processes.³⁰ In addition, binding and catalysis studies on isolated β -subunits have been performed.^{31,32}

Based on these initial findings, further computational investigation of the properties of isolated β -subunits and in different initial conformations with and without nucleotide bound is clearly warranted. Here, we report an investigation of the relaxed configurations of several isolated β subunits with different initial conformations using long unbiased MD simulations (30–120 ns). In our simulations, we observe clear differences in the dynamics of β subunits depending on their initial state. Surprisingly, we also find that the opening of isolated β subunits can take place on a timescale of only several tens of nanoseconds.

The paper is organized as follows. We first outline a general overview of the simulation technique and the systems studied. Subsequently, a scheme for proper structural analysis of the simulation data is described. With these tools in hand, we present the results of our simulations and finally discuss their implications in the context of the fully assembled F_1 -ATPase.

Methods

Over the last several years, a significant number of F_1 -ATPase structures have been determined.⁸ Our computational study uses the β -subunits of the F_1 structure reported in³⁴ (PDB code 1E1R) which was resolved at 2.5 Å. Each of the three β -subunit structures is believed to capture F_1 's ATP synthesis/hydrolysis cycle at a different stage along the rotatory catalytic cycle and thus provides three different β subunit conformations. β_{TP} in 1E1R contains the non-hydrolyzable ATP analogue MgAMP-PNP, β_{DP} contains MgADP, and β_E contains phosphate modeled as $H_2PO_4^-$. Though of minor importance here, we note in passing that the determination of the correct protonation state of the phosphate groups in bound ligands is a complex issue and can certainly modify the outcome of a simulation (see, e.g., Ref. 35). The bound inhibitor molecules ALF_3^- were left out in the simulations.

All-atom MD simulations were performed using the MD program NAMD2³⁶ with the force field CHARMM27.³⁷ This parallel MD code shows very good scalability to a large number of computer cores.^{38,39} The inorganic phosphate ion was parametrized using *ab initio* calculations and resulted in partial charges similar to those given in the supplement of a paper by Yang et al.⁴ Periodic boundary conditions were used and the simulations were carried out in the NPT ensemble using Langevin dynamics (310 K, 1 atm). Furthermore, the particle mesh Ewald (PME) method was employed to evaluate electrostatic forces without cut-off. To constrain the vibrations of hydrogen atoms and therefore to allow the use of a 2 fs time step, the SHAKE algorithm was utilized. Three simulations were performed for each model using different initial conditions, i.e., different initial velocities.

Each of the β subunits simulated in this study was extracted from the corresponding F_1 crystal structure without any initial relaxation of the full complex. The first set of simulations involved the β_E subunit of 1E1R³⁴ and will be referred to as S1 in the following. The protein was solvated in a box of TIP3 water molecules large enough to provide sufficient separation to its periodic images in order to avoid artificial correlations (distances are given in Table 1). The 18-fold negative charge of the β -subunit and the negative charge of the bound $H_2PO_4^-$ ion were compensated by adding 19 randomly placed Na^+ ions. Table 1 provides the molecular composition of system S1. With the protein fixed, 1000 steps of conjugate gradient minimization followed by 100 ps of dynamics were performed to equilibrate the solvent. Then all atoms were released and the system was subject to unbiased MD simulations for another 30 ns.

To investigate the role of the α - β subunit interface on the spontaneous movement of the β -subunits we performed simulation S2. The model consisted of β_E together with its two adjacent α subunits yielding an $\alpha\beta_E\alpha$ -subcomplex. Incidentally, this system contains a complete binding pocket since part of it is actually contributed by the adjacent α -unit. The setup of S2 was identical to the one for S1 and its atom composition is given in Table 1.

A third simulation, S3, started with the closed β_{TP} conformation of 1E1R and included the nucleotide present in the binding pocket as seen in the crystal structure. Since we sought to investigate the behavior of a nucleotide-free but closed β -subunit, we set up an additional simulation S4 in which the ATP present in β_{TP} was removed. Again, details of the simulation setup are given in Table 1.

Analysis procedure

Definition of degrees of freedom

In order to describe the dynamics of isolated β subunits during our unbiased simulations, we defined a set of rigid body coordinates that would capture any opening or closing motions.

The choice was guided by the complete F_1 complex and its pseudo-symmetry axis. Inspection of the simulations showed that the internal motion of the β -subunits is dominated by a hinge bending motion of the C-terminal domains (Gly364 to Ala474), referred to as “jaw” in the following. Based on a visual analysis of the trajectories, we defined the arithmetic mean of the backbone atom positions of residues Gly364, Ser365, and Glu366 in the crystal structure as a hinge point which is assumed to be fixed. A similar choice was used by Cui et al. in their normal mode analysis of F_1 .¹⁴ The hinge bending motion of the C-terminal domain can be described by two angles defined as follows (shown in Figure 2.) A twisting motion described by ϕ_t around an axis parallel to F_1 's pseudo-symmetry axis that runs through the hinge point as well as a bending motion described by ϕ_b defined as the angle between a vector pointing from the hinge point to the DELSEED region (C_α 's of residues 385 to 400) and the rotational axis for the twisting motion (Figure 2). Both ϕ_t and ϕ_b are defined to be zero in the conformation assumed by β_E .

Alignment

Reliable measurement of ϕ_t and ϕ_b during unbiased MD simulations of β subunits requires careful alignment of individual frames. To obtain a reliable alignment procedure, we first established a suitable reference conformation and then extracted a group of C_α atoms whose RMSD with respect to this reference conformation remains small during all our simulations. Individual frames of simulation trajectories were then compared by aligning these C_α atoms to the reference conformation. This procedure removes global translation and rotation and is leaving only internal motions of the protein which were then mapped onto ϕ_t and ϕ_b . The reference conformation itself was determined from the underlying crystal structures. First, β_{TP} and β_{DP} of 1E1R were overlapped with β_E via rotation by 120° and 240° around F_1 's pseudo-symmetry axis, respectively. Then, the average structure of all three conformations was calculated. C_α atoms of β_E which were within $1.5 \text{ \AA}$ of the average structure were chosen to be in the atom selection to which all frames are aligned. This resulted in 232 out of a total of 465 C_α atoms for 1E1R, namely the C_α atoms of residues 9 to 31, 34 to 127, 150 to 152, 180 to 257, 286, 287, 289 to 297, 299 to 309, 324 to 331, 355 to 357, and 359 (see also Figure 3).

Results

Simulation of the open β_E -subunit (S1)

Figure 4 depicts the time evolution of angles ϕ_t and ϕ_b for three independent unbiased MD simulations of β_E . Since we were mainly interested in long-time conformational changes, we employed a 2.5-ns running average to smooth the trajectories. Please note that in all reported simulations no additional forces were applied to the system and all observed changes in conformation were hence intrinsic to the β -subunits, assuming a negligible effect of the environment. The top panel in Figure 4 shows that the twisting angle ϕ_t remains close to its initial value of 0° (corresponding to the conformation of β_E in the crystal structure) during the 30 ns of free dynamics with considerable fluctuations of up to 10° . The horizontal line in the upper panel of Figure 4 indicates an angle of 23.6° corresponding to the conformation found in the closed β subunit, β_{TP} . The lower panel shows the variations in ϕ_b during the unbiased simulations. Similar to ϕ_t , ϕ_b fluctuates around its initial value with no overall change in bending. To quantify the ϕ_t , ϕ_b fluctuations, we averaged the angles over the last 5 ns of all simulations, yielding $\phi_b = 1.8 \pm 2.7^\circ$ and $\phi_t = 5.8 \pm 3.5^\circ$ (see Table 2).

Simulation of the $\alpha\beta_E\alpha$ -subcomplex (S2)

To analyze the impact of environmental effects on the movement of isolated β -subunits, we performed simulation S2 in which the adjacent α -subunits were added back to the system yielding an $\alpha\beta_E\alpha$ -subcomplex. The time evolution of angles ϕ_t and ϕ_b for S2 are shown in

Figure 5. The average angles (last 5 ns) at the end of the simulation were $\phi_b \sim 1.1 \pm 2.1^\circ$ and $\phi_t \sim 5.9 \pm 2.3^\circ$ (see Table 2). Similar to what was seen in S1, the change in the bending angle ϕ_b is again quite small. The twisting angle ϕ_t exhibits a slight tendency toward larger values. Overall, however, the presence of the adjacent α -subunits appears to have little effect on the overall motion of the empty β -subunit which remains structurally close to the initial open conformation found in the crystal structure. This supports the idea that our simulations of isolated β -subunits can be used to explore their intrinsic equilibrium conformations and interpret them in the context of the fully assembled F_1 subunit.

Simulation of the closed β_{TP} subunit with bound nucleotide MgATP (S3)

Using simulation set S3 we explored the closed β_{TP} state with bound nucleotide MgATP in the catalytic site. The time evolution of angles ϕ_t and ϕ_b for S3 is shown in Figure 6. S3's conformation is significantly different from both S1 and S2 as is apparent in the values of ϕ_t and ϕ_b which are now $\phi_t = 23.6^\circ$ and $\phi_b = 21.5^\circ$ at the beginning of the simulation. All three runs exhibit an initial rapid decrease in both ϕ_t and ϕ_b by $\sim 5^\circ$. Following that, all trajectories have a clear tendency towards smaller bending angles. One trajectory actually starts to fluctuate around $\phi_b = 0^\circ$ at a time of around $t = 40$ ns. For the twisting angle the situation is not that clear. One trajectory fluctuates at rather large ϕ_t values between 10° and 23.6° . The other two simulations instead exhibit much lower ϕ_t values at the latest stage of the simulation, i.e., after 100 ns. At the end of the simulations one of the three trajectories reaches conformations which are in the fluctuation range of the open conformation simulation S1. Since it might be slightly surprising that the closed β_{TP} with bound nucleotide MgATP goes to the open conformation, we investigate the latter trajectory in more detail. It exhibits a rather distinct change in ϕ_t toward the closed configuration initiated by a rather sudden conformational change in ϕ_t at around 20 ns followed by partial unbinding of MgATP from the binding pocket (see Figure 7). By the end of the simulation, this trajectory reaches a conformation close to the one in S1 and S2. Since partial unbinding of ATP follows the structural transition of the β subunit it is not the likely trigger for the conformational change. The partial unbinding of ATP itself may be facilitated by the lack of an adjacent α -subunit. Overall, β_{TP} with bound nucleotide has a tendency towards the open conformation (first two trajectories) but appears to have the ability to fully open up (third trajectory) in conjunction with partial nucleotide unbinding from the catalytic site.

Simulation of the closed nucleotide-free β_{TP} -subunit (S4)

In order to investigate the impact of the presence of nucleotide in the binding pocket on the motion of the β_{TP} subunit, we removed the nucleotide from S3, yielding S4. Apart from the presence/absence of nucleotide, initially S3 and S4 were structurally identical. Overall, the behavior shown by S4 during the unbiased MD simulations is similar to what we observe for S3 (see Figure 8). In particular, one of the trajectories behaves differently from the other two and distinctly moves toward the open conformation (both ϕ_t and ϕ_b tend toward 0°). Compared to S3, this opening motion in S4 is more pronounced and nearly complete after 40 ns. However, it is not clear from our data if this is related to the absence of nucleotide or rather is a statistical effect. The remaining two trajectories of S4 appear to fluctuate in ϕ_t similar to their S3 counterparts and also remain closer to their starting value of 23.6° . Only after 80 ns does the average of this angle go to smaller values. This indicates that the presence of nucleotide may actually increase the ability of β_{TP} to fluctuate. In terms of ϕ_b , both S3 and S4 show large fluctuations and a general tendency toward smaller angles. Towards the end of the simulation there are two trajectories with a quite small bending angle ϕ_b . Figure 9 shows a visualization of the changes in sample close to the end of the simulation. The left panel shows the differences between the closed and open conformation based on the crystal structures. As can be seen larger changes are only needed in the jaw region. During the unbiased simulation of individual β subunits the parts which are very

similar in the crystal structure of closed and open structures start to deviate. This is due to the fact that the simulation are on individual β subunits in water without the neighbouring subunits. Therefore, the RMSD of the complete protein is not a very informative measure concerning the domain motion of the protein. It is clearly visible that the residues in the “jaw” region have the largest movements but the residues in the binding pocket also move considerably.

Discussion

We have used unbiased MD simulations of isolated β subunits of F_1 -ATPase to study their equilibrium conformations under different initial conditions. During all simulation runs we observe fairly sizable fluctuations in the angles ϕ_t and ϕ_b . On the other hand, these fluctuations also suggest a rather flat free energy landscape (on the scale of thermal fluctuations) on which the β subunit systems move during the simulations.

The perhaps strongest evidence regarding the equilibrium conformation of isolated β subunits can be gleaned from simulation S1 which clearly shows that the relaxed structure of a nucleotide-free, open β -subunit is the open one. Despite large fluctuations in both ϕ_t and ϕ_b for all three S1 runs both angles remain close to their starting values for the duration of the simulations. Simulations S4 are more difficult to interpret in this regard. They also correspond to a nucleotide-free β subunit, but this time with a closed initial structure. Two of the trajectories (black and green trajectories in Figure 8) do not arrive at the open conformation during the simulated 120 ns but exhibit a complete movement toward the open conformation in terms of one of the two angles. Interestingly, a third trajectory (red in Figure 8) fully opens as evidenced by both ϕ_t and ϕ_b reaching values of around zero at the end of our simulations. This result is quite surprising since it shows that the closed β subunit can go through a complete opening motion on a timescale of 50 ns. Together with the large fluctuations in ϕ_t and ϕ_b observed in all simulations this suggests a steep but smooth energy landscape connecting the closed and open conformation. Since two of the simulations continue to fluctuate around values larger than those of the open conformation, there likely exist small barriers toward the open conformation that these trajectories have yet to overcome. Together, the results for S1 and S4 indicate that the relaxed structure of the nucleotide-free β subunit is the open conformation.

These findings are in agreement with a study by Sun et al.¹³ which investigated the energy stored in the β -sheet as the β -subunit undergoes its hinge bending motion. The authors created a sequence of interpolated structures between the open and the closed state of the β -subunit, refined them using MD and deduced the relative free energies belonging to the β -sheets of the equilibrated structures using umbrella sampling. The obtained free energy profile shows a minimum very near but not identical to the open conformation. The profile is rather flat for about half the number of interpolation points and then shows a steep increase. Such an energy profile fits quite nicely with the finding from our simulations: the steep part of the potential leads to the fast opening motion of the closed nucleotide-free conformation while the flat part causes the relatively large fluctuations in the open conformation. It should be pointed out that the study described in¹³ only investigated the central β -sheet of the β -subunit and not the complete β -subunit. Furthermore, our results are also in good agreement with experimental findings by Yagi et al.²⁹ who conclude that the β -subunit without nucleotides takes the empty form, at least within their experimental accuracy. At the same time the present investigation as well as the studies by Sun et al.¹³ and Yagi et al.²⁹ nicely agree with the recent MD simulations by Ito and Ikeguchi.²⁸ In this study two single 100 ns long MD simulations were performed on the β_E structure of two different species. In the present investigation the simulation for this setup were only 30 ns

long but used 3 samples. Furthermore, in the setup S2 we included the neighbouring α -subunits which did not change the outcome significantly.

The question arises as to what happens to an initially closed subunit which has nucleotide bound. Simulation S3 was intended to shed light on this question but its interpretation turned out to be difficult. Overall, the changes in ϕ_t and ϕ_b seem similar to the ones for S4. However, all trajectories in Figure 6 remain in the twisting angle at values not too far from the closed conformation. At the end of the simulation, one trajectory corresponds to a full opening event like in S4. Hence, the nucleotide-bound β subunit overall seems to be somewhat more closed than its nucleotide-free version, but presence of the nucleotide is clearly not enough to keep it fully closed. This indicates that the open conformation is actually a structural property of the β subunit independent of the presence of nucleotide. An interesting event observed, e.g., in the third trajectory of our S3 simulations (red in Figure 6) is the partial unbinding of ATP following the rapid structural transition visible in ϕ_t after approximately 22 ns. This event could be interpreted as the initiation of the ATP unbinding process one would expect to occur during or after opening of the β subunit. However, since our simulations are lacking the adjacent α -subunits, we can not rule out that this event might be an artifact of our simulation setup. The unbinding of ATP from the tight binding state has also been studied using interpolated atomic structures⁹ by Antes et al.¹²

Experimentally, Yagi et al.²⁹ conclude in their NMR study that the equilibrium conformation of β -subunits with bound nucleotide is the closed conformation. This is not in agreement with our simulations which show a partial unbinding of the nucleotide and opening of the β subunit. A recent experimental study³² shows, for example, substantial energetic effect depending on the type of nucleotide bound, i.e., MgATP, ATP, MgADP, or ADP. A possible explanation for the discrepancy between theory and simulation is that the details of the nucleotide binding are not correctly represented by the present force field approach. For such binding details a QM/MM study as reported in Refs. 19 and 20 would be needed.

Since simulations S1 of the isolated β -subunit and S2 of the $\alpha\beta\alpha$ -complex are rather similar, it seems that movement of the β -subunits is not influenced or hindered considerably by the adjacent α -subunits. This suggests that the behaviour of individual β -subunits reported in the present study will be similar to β -subunits inside a fully assembled F_1 complex. Nevertheless the high charge state of the individual β -subunits suggests that their conformation is influenced significantly by the electrostatic interaction with its environment, particularly with the γ -stalk and especially its jaw.²

In the present simulations the twisting motion within β -subunits during relaxation of the closed conformation towards the open conformation is clockwise. Through a mainly steric coupling to the γ -stalk, this motion would push the stalk into a counterclockwise rotation. This is in agreement with the assumption that, when the hydrolysis products are released from the closed conformation, the recoil of the β -subunit towards the open conformation underlies a counterclockwise, 40° rotational substep observed in experiment.^{40,41} At the same time there is a bending motion during relaxation of the closed conformation towards the open conformation. We think that this part of the opening motion interacts with the α -helix formed by residues 77 to 90 of the γ -stalk and that this interaction then results in an additional source for the counterclockwise motion of the γ -stalk triggered by its asymmetric shape. Furthermore, the spontaneous twisting motion of the open and empty conformations is probably important for resetting the β -subunits for the next cycle.

In the present simulations we observe that all nucleotide-free trajectories tend to a unique stable conformation with a twisting angle close to that of the open conformation and a

bending angle similar to that of β_E . Upon nucleotide binding the bending angle would have to change to that of β_{TP} and this would most probably cause a power stroke resulting in the 80° substep rotation. From the present investigation we can conclude that the closed β -subunits behave like pre-stressed springs which want to open once the nucleotide is unbound. Furthermore the closing seems to mainly be triggered by nucleotide binding^{9,13} with the binding energy stored in hydrogen bonds and the β -sheets.

The present study uses an all-atom structure, no coarse graining techniques were employed. Furthermore the individual β -subunits are allowed to move without any constraint and especially without forcing to a predefined path. No steering or targeted forces are being used during the MD simulations. Almost all previous simulations did apply one or more of the above listed constraints or approximations. Therefore, the current investigation serves as a test and confirmation of results obtained using constrained and/or approximate methods. In addition, interesting dynamical details of the free motion of individual β -subunits, such as the approximate timescale of β -subunit opening, were obtained.

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References

1. Abrahams JP, Leslie AG, Lutter R, Walker JE. *Nature*. 1994; 370:621–628. [PubMed: 8065448]
2. Berzborn RJ, Schlitter J. *FEBS Lett*. 2003; 533:1–8. [PubMed: 12505149]
3. Mao HZ, Weber J. *Proc Natl Acad Sci USA*. 2007; 104:18478–83. [PubMed: 18003896]
4. Yang W, Gao YQ, Cui Q, Ma J, Karplus M. *Proc Natl Acad Sci USA*. 2003; 100:874–879. [PubMed: 12552084]
5. Boyer PD. *Biochemistry (Mosc)*. 2001; 66:1058–1066. [PubMed: 11736627]
6. Boyer PD. *FEBS Lett*. 2002; 512:29–32. [PubMed: 11852046]
7. Senior AE, Nadanaciva S, Weber J. *Biochim Biophys Acta*. 2002; 1553:188–211. [PubMed: 11997128]
8. Weber J, Senior AE. *FEBS Lett*. 2003; 545:61–70. [PubMed: 12788493]
9. Wang H, Oster G. *Nature*. 1998; 396:279–282. [PubMed: 9834036]
10. Oster G, Wang H. *Biochim Biophys Acta*. 2000; 1458:482–510. [PubMed: 10838060]
11. Sun SX, Wang H, Oster G. *Biophys J*. 2004; 86:1373–1374. [PubMed: 14990467]
12. Antes I, Chandler D, Wang H, Oster G. *Biophys J*. 2003; 85:695–706. [PubMed: 12885621]
13. Sun S, Chandler D, Dinner AR, Oster G. *Eur Biophys J*. 2003; 32:676–683. [PubMed: 12955360]
14. Cui Q, Li G, Ma J, Karplus M. *J Mol Biol*. 2004; 340:345–352. [PubMed: 15201057]
15. Zheng W. *Proteins*. 2009; 76:747–752. [PubMed: 19280602]
16. Liu MS, Todd BD, Sadus RJ. *Biochim Biophys Acta*. 2006; 1764:1553–1560. [PubMed: 17010684]
17. Gao YQ, Yang W, Karplus M. *Cell*. 2005; 123:195–205. [PubMed: 16239139]
18. Steinbrecher T, Hücke O, Steigmüller S, Borsch M, Labahn A. *FEBS Lett*. 2002; 530:99–103. [PubMed: 12387874]
19. Dittrich M, Hayashi S, Schulten K. *Biophys J*. 2003; 85:2253. [PubMed: 14507690]
20. Dittrich M, Hayashi S, Schulten K. *Biophys J*. 2004; 87:2954–2967. [PubMed: 15315950]
21. Strajbl M, Shurki A, Warshel A. *Proc Natl Acad Sci USA*. 2003; 100:14834–9. [PubMed: 14657336]
22. Böckmann RA, Grubmüller H. *Nature Struct Biol*. 2002; 9:198.
23. Ma J, Flynn TC, Cui Q, Leslie AGW, Walker JE, Karplus M. *Structure*. 2002; 10:921. [PubMed: 12121647]

24. Tajkhorshid, E.; Aksimentiev, A.; Balabin, I.; Gao, M.; Isralewitz, B.; Phillips, JC.; Zhu, F.; Schulten, K. Large Scale Simulation of Protein Mechanics and Function. In: Richards, FM.; Eisenberg, DS.; Kuriyan, J., editors. *Advances in Protein Chemistry*. Elsevier; New York: 2003.
25. Ito Y, Ikeguchi M. *J Comput Chem*. 2010; 31:2175–2175. [PubMed: 20336770]
26. Schulz R, Vargiu AV, Collu F, Kleinekathöfer U, Ruggerone P. *PLoS Comput Biol*. 2010; 6:e1000806. [PubMed: 20548943]
27. Böckmann RA, Grubmüller H. *Biophys J*. 2003; 85:1482–1491. [PubMed: 12944266]
28. Ito Y, Ikeguchi M. *Chem Phys Lett*. 2010; 490:80–83.
29. Yagi H, Tsujimoto T, Yamazaki T, Yoshida M, Akutsu H. *J Am Chem Soc*. 2004; 126:16632–8. [PubMed: 15600369]
30. Yagi H, Kajiwarra N, Iwabuchi T, Izumi K, Yoshida M, Akutsu H. *J Biol Chem*. 2008; 284:2374 – 2382. [PubMed: 19028677]
31. Jose-Nunez C, Torres-Larios A, Ramirez-Silva L, Mendoza G, Salcedo G, Gomez-Puyou A, Tuena de Gomez-Puyou M. *J Bioenerg Biomembr*. 2009; 40:561–568. [PubMed: 19139978]
32. Pulido NO, Salcedo G, Perez-Hernandez G, Jose-Nunez C, Velazquez-Campoy A, Garcia-Hernandez E. *Biochemistry*. 2010; 49:5258–5258. [PubMed: 20518490]
33. Humphrey WF, Dalke A, Schulten K. *J Mol Graph*. 1996; 14:33–38. [PubMed: 8744570]
34. Braig K, Menz RI, Montgomery MG, Leslie AG, Walker JE. *Structure*. 2000; 8:567–573. [PubMed: 10873854]
35. Banas P, Walter NG, Sponer J, Otyepka M. *J Phys Chem B*. 2010; 114:8701–8702. [PubMed: 20536206]
36. Kalé L, Skeel R, Bhandarkar M, Brunner R, Gursoy A, Krawetz N, Phillips J, Shinozaki A, Varadarajan K, Schulten K. *J Comp Phys*. 1999; 151:283.
37. MacKerell A, et al. *J Phys Chem B*. 1998; 102:3586–3616.
38. Kumar S, Huang C, Zheng G, Bohm E, Bhatele A, Phillips JC, Yu H, Kalé L. *IBM J Res & Dev*. 2008; 52:177–187.
39. Detailed performance and scaling information for a system of similar size can be found. <http://www.ks.uiuc.edu/Research/namd/performance.html>
40. Yasuda R, Noji H, Yoshida M, Kinosita K Jr, Itoh H. *Nature*. 2001; 410:898–904. [PubMed: 11309608]
41. Shimabukuro K, Yasuda R, Muneyuki E, Hara KY, Kinosita K Jr, Yoshida M. *Proc Natl Acad Sci USA*. 2003; 100:14731–6. [PubMed: 14657340]

Figure 1.

F₁-ATPase complex and β -subunit. Left panel: Side view of the F₁-ATPase complex. The backbone atoms of the open β -subunit (left panel in blue), of a closed β -subunit (right panel in blue), and of the γ -stalk (in red) are shown in cartoon representation. The arrow indicates the pseudo-symmetry axis of the entire F₁-ATPase complex. Right panel: Configuration for simulation S1 starting from the open conformation of a β -subunit with the balls denoting sodium ions. All molecular images in this article were created using the molecular graphics program VMD.³³

Figure 2.

Definition of twisting angle ϕ_t and bending angle ϕ_b . The right vertical arrow denotes the rotational axis of the twisting motion being parallel to the pseudo-symmetry axis (left vertical arrow). The bending angle ϕ_b is within the (vertically shown) plane which goes through the left arrow.

Figure 3.

Conformation of β_E : Shown is the backbone of the open β -subunit in cartoon representation together with the C_α atoms which are used for the alignment shown in van der Waals representation. The C-terminal domains (Gly364 to Ala474), referred to as “jaw”, are shown in yellow. The arrow indicates the pseudo-symmetry axis of the entire F_1 -ATPase complex.

Figure 4.

Results of simulation S1 starting from the empty conformation of the β -subunit. The upper panel shows the twisting angle ϕ_t while the lower panel displays the bending angle ϕ_b . Data points have been analyzed every 10 ps and then an running average over 250 frames, i.e. 2.5 ns, has been applied. The dashed lines indicate the results from a linear regression to guide the eye.

Figure 5.
Results of the $\alpha\beta_E\alpha$ -subcomplex simulations S2.

Figure 6. Resulting angles for simulation set S3 starting from the closed confirmation. The initial conformation starts at the horizontal blue lines which is not visible due to the running average over 2.5 ns.

Figure 7. Snapshots from simulation S3: Rapid change in the ATP conformation between 27.8 and 27.9 ns resulting finally in a complete opening of the β -subunit. ATP is shown as van der Waals spheres, the β -subunit as cartoon representation.

Figure 8. Resulting angles for simulation set S4 starting from the closed confirmation but, in contrast to S3, without nucleotide.

Figure 9.

“Porcupine” plots with arrows indicating the differences between two conformations. In the left panel the initial conformation of S4 is shown with the arrows indicating the residue C_{α} displacements to the open crystal structure (the colour scale denotes the displacements in Å). A typical conformation close to the end of an S4 simulation is shown in the right panel together with the arrows indicating the differences to the open crystal structure. Clearly the final conformation of the simulation is getting closer to the open conformation in the “jaw”.

Table 1

Composition of all simulated systems. Given are the crystal structure from which the starting structures were derived, the atom composition, i.e. the number of atoms belonging to certain atom classes, and the minimal distance of the protein from its nearest periodic images.

simulation set	S1	S2	S3	S4
structure	β_E of 1E1R	$\alpha\beta_E\alpha$ of 1E1R	β_{TP} of 1E1R	β_{TP} of 1E1R
total # of atoms	58888	128382	61578	61552
protein	7121	22189	7121	7121
water	51729	106035	54381	54399
H_2PO_4^-	7	7	0	7
ADP	0	0	0	0
ATP	0	86 (in α 's)	43	0
Mg^{2+}	0	2 (in α 's)	1	0
Na^+	25	39	26	25
Cl^-	6	24	6	7
min. dist. [$\text{\AA}$]	22.3	20.4	23.3	23.3

Table 2

Results for the averaged angles (last 5 ns of the corresponding trajectory) and the corresponding standard deviation in brackets.

simulation set	length [ns]	ϕ [°]	ϕ [°]
S1	crystal	0.00	0.00
	30	6.66 (2.60)	3.34 (1.91)
	30	1.22 (4.55)	2.90 (3.55)
	30	9.47 (3.33)	−0.96 (2.63)
	average	5.78 (3.49)	1.76 (2.70)
S2	crystal	0.00	0.00
	30	3.69 (2.82)	2.09 (1.83)
	30	9.23 (1.91)	2.91 (2.10)
	30	4.80 (2.27)	−1.71 (2.26)
	average	5.91 (2.33)	1.10 (2.06)
S3	crystal	22.58	21.52
	120	7.94 (2.32)	9.39 (1.93)
	120	18.04 (3.10)	9.11 (2.25)
	120	7.42 (3.05)	3.81 (1.73)
	average	11.13 (2.82)	7.44 (1.97)
S4	crystal	22.58	21.52
	120	15.04 (5.32)	2.85 (2.20)
	120	0.86 (3.71)	0.57 (2.49)
	120	0.49 (2.71)	9.51 (1.84)
	average	5.46 (3.91)	4.31 (2.18)