Rocking ratchet based on F$_1$-ATPase in the absence of ATP

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Abstract. - Bartussek, Hänggi and Kissner studied a rocking ratchet system, in which a Brownian particle is subject to an asymmetric periodic potential together with an oscillating force, and found that the direction of the macroscopic current can be reversed by changing the parameter values characterizing the model [Europhys. Lett., 28 (1994) 459]. In this letter, we apply their ratchet theory to a rotary motor-protein, F$_1$-ATPase. In this work, we construct a model of a rocking ratchet in which F$_1$-ATPase rotates not as a result of ATP hydrolysis but through the influence of an oscillating force. We then study the motion of F$_1$-ATPase on the basis of molecular dynamics simulations of this coarse-grained protein model. Although in the absence of ATP, F$_1$-ATPase exhibits directionless Brownian motion when there exists no oscillating force, we observe directional motion when we do apply an oscillating force. Furthermore, we observe that the direction of rotation is reversed when we change the oscillation frequency.

Introduction. – Recently, theories of non-equilibrium statistical mechanics have been applied to many biological systems. Among such studies, the fluctuation-dissipation theorem has been investigated with the purpose of understanding the motion of myosins in an actin network [1], the fluctuation theorem has been used to quantify the fluctuations of cell motion [2], and the Jarzynski equality and the Crooks theorem have been applied to RNA hairpins in order to measure their free energies [3]. Recent technical developments have made it possible to investigate small systems, including biological systems, and this has provided another way to test theories of non-equilibrium statistical mechanics in addition to the investigation of theoretical models of stochastic processes.

Ratchet theories have been proposed to describe certain types of non-equilibrium statistical behavior [4–6], and they predict that together, spatial asymmetry and non-equilibrium effect, can cause directional motion in stochastic systems [5]. Among the various ratchet models, that consisting of a Brownian particle in an asymmetric periodic potential together with an oscillating force is called a “rocking ratchet.” For such ratchets, it has been shown that the direction of the current can be reversed by changing the parameter values characterizing the model [6]. Although the application of such current reversal to nano-sized systems is mentioned in Ref. [6], as far as we know, there is yet no such experiment. In this letter, we numerically investigate the rocking ratchet theory in application to a nano-sized bio-molecule to which many theories of non-equilibrium statistical mechanics have been applied in recent years.

We study a rotary motor-protein, F$_1$-ATPase, in which a γ-subunit rotates in the center of α$_3$β$_3$-subunits [7, 8]. In this letter, we construct a model of a rocking ratchet in which F$_1$-ATPase rotates through the influence of an oscillating force, and particularly study the motion of F$_1$-ATPase in the absence of ATP. Because in this case no nucleotide is attached to the β-subunits, such a state is called a nucleotide-free state. In the absence of ATP, it was observed that the γ-subunit exhibits directionless Brownian motion with rotational steps of ±120° [9]. This indicates that the rotational potential experienced by a γ-subunit is periodic with a period of 120° reflecting the three-fold symmetry of α$_3$β$_3$-subunits. Then, if this periodic potential, which reflects the interaction between a γ-subunit and α$_3$β$_3$-subunits, is asymmetric, we expect that a γ-subunit will display a directional rotation when we apply an oscillating force.

In order to test this conjecture, we performed molecular dynamics simulations of a protein model, in which a protein molecule is regarded as consisting of α-carbon atoms, employing a coarse-grained representation of the amino-acid residues [10]. We find that the γ-subunit ro-
tates counterclockwise when viewed from the membrane side in the case that the oscillation period is larger than the characteristic time scale of the system dynamics, and it rotates clockwise in the opposite case.

**F$_1$-ATPase in the presence of ATP.** – F$_1$-ATPase is a rotary motor-protein in which a $\gamma$-subunit rotates in the center of $\alpha_3\beta_3$-subunits due to ATP hydrolysis [7,8]. In the presence of ATP, a $\gamma$-subunit rotates counterclockwise when viewed from the membrane side and makes a 120° step as a result of the consumption of one ATP molecule. (In this letter, the counterclockwise direction is regarded as the plus direction.) Each step of 120° is divided into 40° and 80° substeps, caused by the two chemical reactions involved in this process, i.e. the product release and the ATP binding, respectively.

X-ray studies of the crystal structure of F$_1$-ATPase reveal that a $\beta$-subunit possesses several different structures [11]. The ATP-bound $\beta$-subunit, the ADP-bound $\beta$-subunit and the nucleotide-free $\beta$-subunit are denoted by $\beta_{TP}$, $\beta_{DP}$ and $\beta_E$, respectively. While $\beta_{TP}$ has a closed form at its C terminus, $\beta_E$ has an open form. Because the structures of the $\beta$-subunits change as the chemical reactions involved in ATP hydrolysis proceed, it is widely believed that the coordinated push-pull motion of the C termini of $\beta$-subunits causes the rotation of the $\gamma$-subunit [12]. Such substeps have been observed in numerical studies of coarse-grained protein models by changing the reference structures of the $\beta$-subunits so as to create the push-pull motion [13,14]. In this letter, although we do not study such push-pull motion, we do study rotation in F$_1$-ATPase. This rotation is realized in the absence of ATP through use of an oscillating force in a coarse-grained protein model (see the Appendix).

**F$_1$-ATPase in the absence of ATP.** – While a $\gamma$-subunit always makes steps of +120° in the presence of ATP, both forward, +120°, and backward, −120°, steps can be observed experimentally in the absence of ATP [9]. Because the structure of $\beta_E$, which has an open form, makes the height of the rotational potential smaller [9], both +120° and −120° steps can be observed.

First, we investigated the Brownian motion of F$_1$-ATPase in equilibrium (without an oscillating force) through numerical simulations. Here, we assume that the reference structure of the $\alpha_3\beta_3$-subunits in a nucleotide-free state can be represented by $\{\alpha_E\beta_E, \alpha_E\beta_E, \alpha_E\beta_E\}$, and that in equilibrium there is no structural change of the $\beta$-subunits. We computed the time evolution of the rotation angle $\theta(t)$, which represents the direction of the $\alpha$-carbon atoms in the $\gamma$-subunit encircled in the top-left region of Fig. 3. (See the Appendix and Ref. [13] for a precise definition.) In the upper graphs of Fig. 2, we plot a typical trajectory of $\theta(t)$ in the nucleotide-free state as a function

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Fig. 1: Schematics of the F$_1$-ATPase in a nucleotide-free state, where the F$_1$-ATPase consists of the $\alpha$-subunits (light gray), the $\beta$-subunits (gray) and the $\gamma$-subunit (black). $\{\alpha_E\beta_E, \alpha_E\beta_E, \alpha_E\beta_E\}$ is composed of the $\alpha_E\beta_E$-subunits of the crystal structure 1BMF [11].

Fig. 2: (Top) An example trajectory of the angle, $\theta$, of the $\gamma$-subunit in a nucleotide-free state. $\theta = 0°$ represents the position of the $\gamma$-subunit in the crystal structure 1BMF [11]. The dotted lines represent $\theta = 20° + 120°n \ (n = 0, \pm 1, \pm 2\cdots)$. (Bottom) The rotational potential, $-\log(P(\theta))$. (Inset) The time correlation function, $\langle\theta(t)\theta(0)\rangle - \langle\theta(0)\rangle^2$, in dwell time (circles). The fitting curve (a thick black curve) represents $2.2 \exp(-t/5, 236\Delta t)$.  

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Fig. 3: (Top) Schematics of the rocking ratchet based on F$_1$-ATPase in a nucleotide-free state. (Bottom) In the case that there exists an oscillating force with $\tau_\Omega = 0.005\Delta t$ and $A = 0.005$, the $\gamma$-subunit exhibits counterclockwise rotation. The snapshots were taken using VMD [15].

of time. Directionless Brownian motion is observed, as there are both steps of $+120^\circ$ and $-120^\circ$. In the upper graphs of Fig. 2, it is seen that the $\gamma$-subunit stops at angles of approximately $20^\circ + 120^\circ n$ ($n = 0, \pm 1, \pm 2, \cdots$). We note that it has also been observed experimentally that in the absence of ATP, the $\gamma$-subunit stops at angles shifted by $-20^\circ$ from the ATP binding angle [9]. Because the ATP binding angle is considered to be $\theta = 30^\circ - 40^\circ$ in our model [13], the stopping angles plotted in the upper graphs of Fig. 2 seem to correspond to the experimental results [9].

In order to investigate the system dynamics in the intra-well of the rotational potential, we analyzed trajectories of $\theta(t)$ that is fluctuating around an angle of $20^\circ + 120^\circ n$ ($n = 0, \pm 1, \pm 2, \cdots$). By using the probability distribution, $P(\theta)$, we compute the rotational potential $-\log(P(\theta))$. Then, its asymmetric profile is seen in the lower graphs of Fig. 2. In the inset of the lower graphs of Fig. 2, we further plot the time correlation function, $(\langle \theta(t)\theta(0) \rangle - \langle \theta(0) \rangle^2)$. From the fitting curve, the intra-well relaxation time is estimated to be $5,236\Delta t$.

**Rocking ratchet.** Having verified that our protein model appears to be capable of describing the motion of F$_1$-ATPase in the absence of ATP, we next investigated the case in which an oscillating force is applied to the $\gamma$-subunit in order to create a rocking ratchet. This force mimics one that can be applied by a magnetic tweezers (Fig. 3). Explicitly, we apply the oscillating force

$$f_x = (A \sin 2\pi t/\tau_\Omega \sin \theta, -A \sin 2\pi t/\tau_\Omega \cos \theta)$$  \hspace{1cm} (1)

to the $\alpha$-carbon atoms in the $\gamma$-subunit that are not inside the $\alpha_3$/$\beta_2$-subunits (marked in red in Fig. 3). Note that although in real experiments an oscillating force should be applied to the bead to which a $\gamma$-subunit is attached, for simplicity, here we apply this force directly to the $\alpha$-carbon atoms in the $\gamma$-subunit. In the lower graphs of Fig. 4, we plot typical trajectories in the case with an oscillating force of periods $\tau_\Omega = 0.005\Delta t$ and $\tau_\Omega = 4,000\Delta t$, respectively. In Fig. 4, it is seen that when $A = 0.005$, the range of oscillation of the $\gamma$-subunit is approximately $120^\circ$ which is the period of the potential, and that the $\gamma$-subunit is likely to stop at the same angles as in equilibrium even in the case with the oscillating force.

In Fig. 5, we plot 20 trajectories in the cases $\tau_\Omega = 50,000\Delta t$ and $\tau_\Omega = 4,000\Delta t$. There, we can see clearly that rotation has a directional preference. Interestingly, the average direction of rotation in the case with $\tau_\Omega = 50,000\Delta t$ is opposite to that in the case $\tau_\Omega = 4,000\Delta t$. It is thus seen that current reversal occurs when we change the oscillation period. Comparing these results with the directions of rotation found in Ref. [6], they lead us to conjecture that the slope of the potential in the system studied here, like that studied there, is somewhat smaller in the counterclockwise direction (the plus direction) than that in the clockwise direction. Indeed, the rotational potential plotted in the lower graphs of Fig. 2 is consistent with this conjecture.

In order to determine the value of $\tau_\Omega^*$ at which the current reversal occurs, in Fig. 6, we plot the average currents $\langle \theta(t_f)/\tau_f \rangle$ as functions of $\tau_\Omega$ for the several values of the oscillation amplitude. (Here, $\tau_f = 2 \times 10^6\Delta t$.) We find...
that the current vanishes at a value \( \tau^*_\Omega \sim 6,000\Delta t \). Although we do not yet have a complete understanding of the mechanism of the current reversal, we believe that \( \tau^*_\Omega \) is related to the intra-well relaxation time in equilibrium, which is estimated to be 5,236\( \Delta t \) (see the inset of the lower graphs of Fig. 2). In Ref. [6], it is asserted that the intra-well relaxation time determines the oscillation period, which causes the current reversal in a rocking ratchet system. Taking these into consideration, the current reversal observed in our protein model seems to be similar to that phenomenon originally reported in Ref. [6].

**Discussion.** – In conclusion, we investigated motion of F\(_1\)-ATPase in the absence of ATP by performing numerical simulations of the coarse-grained protein model, and fabricated a rocking ratchet applying the ratchet theory studied in Ref. [6] to F\(_1\)-ATPase. We observed the current reversal when we changed the oscillation frequency and predicted the directions of rotation when there exists an oscillating force. We hope that our predictions for a rocking ratchet consisting of F\(_1\)-ATPase in a nucleotide-free state will be confirmed experimentally, and that combination of theories of non-equilibrium statistical mechanics with protein simulations will yield new understanding of statistical properties possessed by proteins [10,16]. Below, we discussed two things related to our results.

It should be noted that the parameter values used in our simulation do not correspond to room temperature and the real time scale of experiments. The temperature used in our simulations corresponds to one third less than the room temperature in order for the \( \gamma \)-subunit not to lose its shape when the oscillating force is applied. However, we believe that the basic prediction of our model - that the direction of rotation can be reversed by altering the period of the oscillating force - would be unchanged if such parameter values were used. In previous numerical studies [13, 14] also, temperatures much less than room temperature were used in order to make the coupling between the structure change of the \( \beta \)-subunits and the rotation of the \( \gamma \)-subunit “tight,” on the basis of the idea of the push-pull model [12]. However, it is worth simulating the motion of F\(_1\)-ATPase with realistic parameter values that represent the physiological conditions in which the molecular softness and thermal fluctuations would be more important. With such conditions in mind, the ratchet-based mechanism has been considered instead of the push-pull model [17] in an attempt to account for the results of a recent experiment [18]. The same idea has been applied to an F\(_0\)-motor system [17].

One reason for investigating systems like that considered here is to gain an understanding of motor proteins that could lead to the design and fabrication of new nanomachines that can operate efficiently in thermal noise. For this purpose, it is important to be able to cause directional motion of proteins not through use of ATP hydrolysis but by other energy sources. For this reason, we have studied a system in which the fluctuations and softness of F\(_1\)-
ATPase are exploited in such a manner to cause rotation through the influence of an oscillating force. Because the ratchet theories \[4\]–\[6\] are regarded as providing a theoretical foundation for the style of nanomachines, we hope that this study provides a basis for the development of one class of such machines.

**Appendix.** – Here we describe the model studied in this work. The position and velocity of the \(i\)th \(\alpha\)-carbon atom in \(F_1\)-ATPase are denoted by \(r_i\) and \(v_i\). The Hamiltonian is given by \(H (\{r_i, v_i\}) = \sum_{i=1}^{N} m_i v_i^2 / 2 + V_{\alpha\beta_3} + V_r + V_{\text{int}} + V_{\text{fix}}, \) where \(m\) is the mass of the \(i\)th \(\alpha\)-carbon atom and \(N\) and \(n\) are the number of \(\alpha\)-carbon atoms in the \(\alpha_3\beta_3\)-subunits and in the \(\gamma\)-subunit, respectively. The quantity \(V_{\alpha\beta_3}\) is the elastic network potential for the \(\alpha_3\beta_3\) subunits and is given by

\[
\begin{align*}
V_{\alpha\beta_3}(\{r_i\}_{\alpha\beta_3}) &= \sum_{i=1}^{N} \left( \frac{k}{2} \right) \left( |r_i - r_{i+1}| - |r_i^0 - r_{i+1}^0| \right)^2 \\
&+ \sum_{i=1}^{N} \frac{5k}{2} \left( |r_i - r_{i+2}| - |r_i^0 - r_{i+2}^0| \right)^2 \\
&+ \sum_{i=1}^{N-1} \sum_{j=i+3}^{N} \frac{0.1k}{2} \left( |r_i - r_j| - |r_i^0 - r_j^0| \right)^2,
\end{align*}
\]

where \(\{r_i^0\}\) represents the reference structure, and we have \(k = 0\) when \(|r_i^0 - r_j^0| > r_c\). The elastic network potential, \(V_r\), is defined similarly to \(V_{\alpha\beta_3}\). Here, \(\{r_i^0\}_{\alpha\beta_3}\) is composed of the \(\alpha_\beta_3\)-subunits of the crystal structure 1BMF \[11\], which was taken from the RSCB Protein Data Bank \[19\]. We rotate them by \(\pm 120^\circ\) in the \(xy\)-plane and choose the reference structure as \(\{\alpha_\beta_3, \alpha_\beta_3, \alpha_\beta_3\}\), where the \(xy\)-plane is defined so that the three \(\beta_3\) are on the plane. The set \(\{r_i^0\}\) is also constructed using 1BMF. The interaction potential between the \(\gamma\)-subunit and the \(\alpha_3\beta_3\)-subunits consists of a repulsive potential and an electrostatic potential:

\[
\begin{align*}
V_{\text{int}}(\{r_i\}) &= \sum_{i=1}^{N} \sum_{j=N+1}^{N+n} \left\{ \varepsilon_1 \left( \frac{D}{|r_i - r_j|} \right)^{12} + \varepsilon_2 \left( \frac{g_i g_j}{|r_i - r_j|^2} \right) \right\}.
\end{align*}
\]

Here, \(g_i\) represents the sign of the electric charge of the amino acid around the \(i\)th \(\alpha\)-carbon atom: \(g_i = 1\) when the amino acid is lysine or arginine, \(g_i = -1\) when it is glutamic acid or aspartic acid, and \(g_i = 0\) otherwise. In our model, \(\gamma_{272}\) and the three \(\beta_3\) are fixed by including the potential \(V_{\text{fix}}(r_i) = k_h |r_i - r_i^0|^2 / 2\).

The time evolution of the \(i\)th \(\alpha\)-carbon atom is described by an over-damped Langevin equation with friction coefficient \(\Gamma\) and temperature \(T\). In our simulation, the parameters are set to be \(m = 1, k = 10, N = 2823, n = 122, r_c = 10, D = 6, \Gamma = 0.01, \varepsilon_1 = 3.0, \varepsilon_2 = 0.05\) and \(k_h = 0.5\). We chose these values so that the results obtained without the oscillating force are consistent with the results obtained experimentally \[9\].

Regarding the definition of the rotation angle \(\theta(t)\), as is done in the previous study \[13\], we consider the projection \(\lambda_{xy}(t) = \sum_{i=70}^{90} |r_i(t) - r_{75}(t)| / |r_i(t) - r_{75}(t)|\) onto the \(xy\)-plane. \(\theta(t)\) is defined by the angle between \(\lambda_{xy}(t)\) and \(\lambda_{xy}^{\text{1BMF}}\) where \(\lambda_{xy}^{\text{1BMF}}\) is the corresponding vector calculated for 1BMF \[11\].

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