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Fluctuation theorems and the nonequilibrium thermodynamics of molecular motors

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The fluctuation theorems for the currents and the dissipated work are considered for molecular motors which are driven out of equilibrium by chemical reactions. Because of the molecular fluctuations, these nonequilibrium processes are described by stochastic models based on a master equation. Analytical expressions are derived for the fluctuation theorems, allowing us to obtain predictions on the work dissipated in the motor as well as on its rotation near and far from thermodynamic equilibrium. We show that the fluctuation theorems provide a method to determine the affinity or thermodynamic force driving the motor. This affinity is given in terms of the free enthalpy of the chemical reactions. The theorems are applied to the F_1 rotary motor which turns out to be a stiff system typically functioning in the nonlinear regime of nonequilibrium thermodynamics. We show that this nonlinearity confers a robustness to the functioning of the molecular motor.

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I. INTRODUCTION

Thanks to the recent advances in biophysics, it is nowadays possible to observe the dynamics of single biomolecules such as the molecular motors. Experiments have been devoted to linear motors such as the actin-myosin or the kinesin-microtubule motors, as well as to rotary motors such as the F_oF_1-ATPase and bacterial flagellar motors. These motors are powered by adenosine triphosphate (ATP) or proton currents across a membrane [1]. These molecular motors take part to the cellular metabolism and are therefore working under nonequilibrium conditions. A major preoccupation today is to understand the nonequilibrium thermodynamics of these motors. Because of their nanometric size and their incessant exchanges with their environment, they are exposed to molecular fluctuations and their behavior is thus stochastic as observed experimentally. Accordingly, their motion is unidirectional only on average and random steps in the direction opposite to their mean motion can occur. The mean motion stops at the thermodynamic equilibrium. When the chemical fuel is in excess with respect to its equilibrium concentration, the motor is driven out of equilibrium and its random motion shows a privileged direction on average. The dependence of the mean motion on the chemical concentrations of the reactants and products is a problem of nonequilibrium statistical thermodynamics in the presence of the chemical reactions. According to thermodynamics, out-of-equilibrium chemical reactions are characterized by the concept of affinity introduced by De Donder for macroscopic systems [2]. We may wonder whether such concepts from thermodynamics are still relevant for nanometric motors which are affected by the thermal fluctuations.

The purpose of the present paper is to develop the nonequilibrium statistical thermodynamics of molecular motors and to show that the affinities of the chemical reactions powering the motor can be determined from the fluctuations of the motion of the motor. The affinities are the thermodynamic forces driving the motor and are therefore central quantities for the nonequilibrium thermodynamics of the motor. Here, we propose a method to obtain experimentally these quantities. This method is based on the fluctuation theorems we have recently derived for nonequilibrium chemical reactions [3–6]. The fluctuation theorems state that the ratio of the probabilities for forward and backward displacements is equal to the exponential of the entropy irreversibly produced during a given time interval. This entropy production is related, on the one hand, to the work dissipated and, on the other hand, to the currents and affinities of the irreversible processes taking place in the motor. Originally, fluctuation theorems have been formulated for mechanical systems [7–14], but we have recently been able to extend them to chemical systems [3–6]. On this ground, we here consider the molecular motors which are mechanochemical systems. We show that the fluctuation theorems can be used to determine the affinity or thermodynamic force acting on the motors.

Beside the general theory, we study in detail the F_1 rotary motor [15–21]. Its stator is composed of six proteins. Three of them catalyze the hydrolysis of ATP, which drive the rotation of a shaft. An actin filament or a bead can be glued to this shaft. *In vivo*, the shaft of this F_1 complex is glued to a proton turbine known as F_o which is located in the internal membrane of mitochondria. The whole F_oF_1-ATPase synthetize ATP from proton currents across the membrane. The F_1 protein complex can function in reverse by using the chemical energy of ATP and serve as a motor which performs mechanical work. Such rotary motors can be modeled as stochastic processes including the diffusive rotation of the shaft and the random jumps between the chemical states [15–17,22]. Such processes describe the motion as a succession of random jumps occurring between the different possible orientations of the shaft and the chemical states of the motor. The stochastic description is a suitable framework to take into account the molecular fluctuations which affect not only the mechanical motion of the shaft but also the chemical reactions. Indeed, the reactants and products enter and exit the motor at random times. The reduction of the more complete description can be envisaged if the rotation shows discrete steps and substeps so that the shaft has fast motions between well-defined orientations corresponding to the chemical states of the motor. In this case, we may introduce a stochastic process based on discrete states. Because of their stochasticity, we can only know the transition rates of the random jumps between the discrete states. These transition rates depend on the chemical concentrations according to the mass action law of chemical kinetics [23]. This simple model allows us to obtain various analytical results and describes successfully the behavior of motors. Furthermore, this formulation allows us to derive fluctuation theorems and develop the nonequilibrium statistical thermodynamics of molecular motors.

We first consider a fluctuation theorem for the entropy production [3]. This allows us to study the fluctuations of the work dissipated by the irreversible processes. The connection to Jarzynski's nonequilibrium work theorem [24] is discussed. Next, we use the fluctuation theorem for the currents [4–6], which here correspond to the rotation rate of the motor shaft and to the rates of ATP consumption and adenosine diphosphate (ADP) release by hydrolysis. Since this fluctuation theorem concerns the nonequilibrium fluctuating currents, we can study the dependence of the mean currents on the affinities provided by the difference of chemical potentials and determine if the molecular motor functions in the linear or nonlinear regimes of nonequilibrium thermodynamics. Finally, we obtain estimations of the time necessary in order to observe random rotations in the direction opposite to the mean motion as described by the fluctuation theorems. Our analysis is complementary to the work reported in Ref. [25] especially about the chemical aspects and because we here give exact analytical expressions (in particular, for the generating functions of the large deviations) and quantitative results concerning the F_1 motor.

The plan of the paper is as follows. In Sec. II, we give a summary of the stochastic description along with the two fluctuation theorems. In Sec. III, we introduce a stochastic model describing the rotary molecular motors. The fluctuation theorems are shown to apply and analytical results are obtained. In Sec. IV, we study the case of the F_1 motor. Conclusions are drawn in Sec. V.

II. STOCHASTIC DESCRIPTION AND FLUCTUATION THEOREMS

In the stochastic description, we are interested in the probability $P(\sigma,t)$ to find the system in a state σ at time t. This probability obeys the master equation

$$\frac{dP(\sigma,t)}{dt} = \sum_{\rho,\sigma'} [W_\rho(\sigma'|\sigma)P(\sigma',t) - W_{-\rho}(\sigma|\sigma')P(\sigma,t)]. \tag{1}$$

Such a master equation is known to describe molecular fluctuations down to the nanoscale [23]. An H theorem can be derived for this master equation by introducing the quantity $S(t)\equiv\Sigma_\sigma P(\sigma,t)S^0(\sigma)-\Sigma_\sigma P(\sigma,t)\ln P(\sigma,t)$. The identification of this quantity with the entropy of the system should be validated by agreement with experiments. For macroscopic systems, this justification has been carried out by comparison with the known thermodynamics. This identification is here adopted as a working hypothesis. The entropy is defined in the units of Boltzmann's constant k_B. The master Eq. (1) rules the time evolution of this entropy. Its time derivative dS/dt can be separated into an entropy flux and an entropy production. The H theorem is that this entropy production is always non-negative [26,27]. Here, we are interested in the stationary state where the probabilities become time independent, $dP_{\rm st}(\sigma)/dt=0$. In such nonequilibrium steady states, the entropy production is given by

$$\left.\frac{d_{\rm i}S}{dt}\right|_{\rm st} = \frac{1}{2}\sum_{\rho,\sigma,\sigma'} J_\rho(\sigma,\sigma')A_\rho(\sigma,\sigma') \geq 0 \tag{2}$$

in terms of the mesoscopic currents

$$J_\rho(\sigma,\sigma') \equiv P_{\rm st}(\sigma)W_\rho(\sigma|\sigma') - P_{\rm st}(\sigma')W_{-\rho}(\sigma'|\sigma) \tag{3}$$

and the mesoscopic affinities

$$A_\rho(\sigma,\sigma') \equiv \ln\frac{P_{\rm st}(\sigma)W_\rho(\sigma|\sigma')}{P_{\rm st}(\sigma')W_{-\rho}(\sigma'|\sigma)}. \tag{4}$$

The entropy production vanishes if and only if the conditions of detailed balance

$$P_{\rm eq}(\sigma)W_\rho(\sigma|\sigma') = P_{\rm eq}(\sigma')W_{-\rho}(\sigma'|\sigma) \tag{5}$$

are satisfied for all ρ,σ,σ', which defines thermodynamic equilibrium.

A. The fluctuation theorem for the dissipated work

The random process is a sequence of random jumps occurring at successive times $0<t_1<t_2<\cdots<t_n<t$ and forming a *history* or *path*

$$\Sigma(t) = \sigma_0\xrightarrow{\rho_1}\sigma_1\xrightarrow{\rho_2}\sigma_2\xrightarrow{\rho_3}\cdots\xrightarrow{\rho_n}\sigma_n. \tag{6}$$

During this path, the lack of detailed balance can be characterized by considering the quantity:

$$Z(t) \equiv \ln\frac{W_{\rho_1}(\sigma_0|\sigma_1)W_{\rho_2}(\sigma_1|\sigma_2)\cdots W_{\rho_n}(\sigma_{n-1}|\sigma_n)}{W_{-\rho_1}(\sigma_1|\sigma_0)W_{-\rho_2}(\sigma_2|\sigma_1)\cdots W_{-\rho_n}(\sigma_n|\sigma_{n-1})}. \tag{7}$$

This quantity fluctuates in time and the generating function of its statistical moments is defined by

$$q(\eta) \equiv \lim_{t\to\infty} -\frac{1}{t}\ln\langle e^{-\eta Z(t)}\rangle, \tag{8}$$

where $\langle\cdot\rangle$ denotes the statistical average with respect to the stationary probability distribution of the nonequilibrium steady state. All the moments of the quantity (7) can be recovered by multiple differentiations with respect to the parameter η at the value $\eta=0$. This generating function can be obtained as the maximal eigenvalue $\hat{L}_\eta\vec{g}_\eta=-q(\eta)\vec{g}_\eta$, of the operator

$$(\hat{L}_\eta\vec{g})(\sigma) \equiv \sum_{\rho,\sigma'} [W_{+\rho}(\sigma'|\sigma)^\eta W_{-\rho}(\sigma|\sigma')^{1-\eta}g(\sigma') - W_{-\rho}(\sigma|\sigma')g(\sigma)] \tag{9}$$

as shown by Lebowitz and Spohn [11].

The generating function (8) obeys the fluctuation theorem

$$q(\eta) = q(1-\eta) \tag{10}$$

as a consequence of the microreversibility [3,5,11]. The generating function (8) identically vanishes $q(\eta)=0$ at thermodynamic equilibrium where the conditions of detailed balance are satisfied. A further property is that the mean entropy production of the reaction in the nonequilibrium steady state is given by

$$\left.\frac{d_{\mathrm{i}}S}{dt}\right|_{\mathrm{st}} = \frac{dq}{d\eta}(0) = \lim_{t\to\infty} \frac{1}{t}\langle Z(t)\rangle_{\mathrm{st}} \geq 0. \tag{11}$$

The symmetry of the generating function (8) is related to a large deviation property of the probability distribution of $Z(t)/t$ in the following way [3,11]:

$$\frac{\mathrm{Prob}\left[\frac{Z(t)}{t} \in (\zeta, \zeta + d\zeta)\right]}{\mathrm{Prob}\left[\frac{Z(t)}{t} \in (-\zeta, -\zeta + d\zeta)\right]} \simeq e^{\zeta t} \quad (t \to \infty), \tag{12}$$

which is the usual form of the fluctuation theorem.

In order to obtain the thermodynamic interpretation of the quantity $Z(t)$, we notice that the ratio of the forward and backward transition rates of an elementary process $\sigma \underset{-\rho}{\overset{\rho}{\rightleftharpoons}} \sigma'$ is given by

$$\frac{W_\rho(\sigma|\sigma')}{W_{-\rho}(\sigma'|\sigma)} = e^{\beta(K_\sigma - K_{\sigma'})}, \tag{13}$$

where K_σ is the thermodynamic potential of the state σ and $\beta=(k_{\mathrm{B}}T)^{-1}$ is the inverse temperature. The relations (13) hold if the transitions $\sigma \underset{-\rho}{\overset{\rho}{\rightleftharpoons}} \sigma'$ are slow enough that the system has the time to settle into quasiequilibrium states σ characterized by some thermodynamic potential K_σ. This corresponds to the assumption of local thermodynamic equilibrium. Since chemical reactions take place inside the molecular motor, an adequate thermodynamic potential is the grand-canonical potential or reduced free energy $J=E-TS-\sum_{i=1}^{c}\mu_i N_i = F-\sum_{i=1}^{c}\mu_i N_i$ in the case of isothermal-isochoric-isopotential processes where the volume is fixed as well as the temperature T and the chemical potentials μ_i of the different molecular species X_i. For dilute solutions, the chemical potentials are related to the concentrations by $\mu_i = \mu_i^0 + k_{\mathrm{B}}T \ln([\mathrm{X_i}]/c^0)$ where c^0 is a standard reference concentration. In the case of isothermal-isobaric-isopotential processes with the pressure fixed instead of the volume, the appropriate thermodynamic potential is the reduced free enthalpy $K=E-TS+PV-\sum_{i=1}^{c}\mu_i N_i = G-\sum_{i=1}^{c}\mu_i N_i$. We remark that this potential is not identically vanishing because the system is not homogeneous so that Euler's thermodynamic relations here do not apply. According to Eq. (13), the quantity (7) is given by

$$Z(t) = \ln \prod_{j=1}^{n} \frac{W_{\rho_j}(\sigma_{j-1}|\sigma_j)}{W_{-\rho_j}(\sigma_j|\sigma_{j-1})} = \beta \sum_{j=1}^{n} (K_{\sigma_{j-1}} - K_{\sigma_j})$$
$$= \beta(K_{\sigma_0} - K_{\sigma_n}) = \beta\tau_{\mathrm{ext}}\Delta\theta + \beta\sum_{i=1}^{c}\mu_i \Delta N_i \tag{14}$$

if an external torque τ_{ext} is applied to the shaft of the motor and if $\Delta\theta$ is the increase of its angle during the time interval t. We denote ΔN_i the number of molecules of the species i entering the motor during the same time interval. ΔN_i is positive for the reactants and negative for the products. Equation (14) can be rewritten as

$$Z = \beta(\mathcal{W} - \Delta\mathcal{G}) = \beta\mathcal{W}_{\mathrm{diss}}, \tag{15}$$

where $\mathcal{W}=\tau_{\mathrm{ext}}\Delta\theta$ is the work performed on the system by the external torque, while $\Delta\mathcal{G} = -\sum_{i=1}^{c}\mu_i\Delta N_i$ is the change of free enthalpy in the whole system including the reservoirs of molecules. The difference between the work performed on the system and its change of chemical free enthalpy is the work dissipated by the irreversible processes.

Now, we notice that the generating function of the quantity $Z(t)$ vanishes at $\eta=0$ and $\eta=1$ by the fluctuation symmetry (10) so that we find

$$\langle e^{-Z(t)}\rangle = \langle e^{-\beta(\mathcal{W}-\Delta\mathcal{G})}\rangle \sim 1 \quad \text{for } t \to \infty, \tag{16}$$

which is analog to Jarzynski's nonequilibrium work theorem [24]. A consequence of the inequality $\langle e^x\rangle \geq e^{\langle x\rangle}$ is the inequality

$$\langle \mathcal{W}\rangle \geq \langle \Delta\mathcal{G}\rangle \tag{17}$$

for the work performed on the system. We recover the Carnot-Clausius inequality giving the maximum possible work performed by the motor

$$\langle \Delta G\rangle = \sum_{i=1}^{c}\mu_i\langle\Delta N_i\rangle \geq \langle \mathcal{W}_{\mathrm{motor}}\rangle \tag{18}$$

since $\mathcal{W}_{\mathrm{motor}} = -\mathcal{W}$ and the chemical free enthalpy consumed by the motor is $\Delta G = -\Delta\mathcal{G}$. The inequality (18) is the analog of Carnot inequality here for motors working under isothermal-isobaric-isopotential conditions. The equality is reached for a motor functioning arbitrarily close to equilibrium.

In a nanomotor, the dissipated work (15) fluctuates because of the thermal noise, obeying the fluctuation theorem (12).

B. The fluctuation theorem for the currents

Another far-from-equilibrium relation has been derived recently [4,5]. It concerns the currents crossing the system in the nonequilibrium steady state. Indeed, the nonequilibrium affinities or thermodynamic forces A_γ driving the system out of equilibrium generate currents $j_\gamma(t)$ of heat or particles. The fluctuations of these nonequilibrium currents obey a symmetry relation given by

$$\frac{\mathrm{Prob}\left[\left\{\frac{1}{t}\int_0^t j_\gamma(t')dt' = \alpha_\gamma\right\}\right]}{\mathrm{Prob}\left[\left\{\frac{1}{t}\int_0^t j_\gamma(t')dt' = -\alpha_\gamma\right\}\right]} \simeq e^{\Sigma_\gamma A_\gamma \alpha_\gamma t} \quad (t \to \infty). \tag{19}$$

As before we can introduce the generating function of these currents in order to study their fluctuations

$$Q(\{\lambda_\gamma\};\{A_\gamma\}) = \lim_{t\to\infty} -\frac{1}{t}\ln\langle e^{-\Sigma_\gamma \lambda_\gamma \int_0^t dt' j_\gamma(t')}\rangle. \tag{20}$$

In the nonequilibrium steady state, the mean current of the process γ is given by

$$J_\gamma = \left.\frac{\partial Q}{\partial \lambda_\gamma}\right|_{\{\lambda_\epsilon=0\}} \tag{21}$$

and the higher-order moments can be obtained by successive differentiations, which shows that the function (20) generates the statistical moments of the currents. The symmetry (19) of the fluctuations is then reflected into a symmetry of the generating function

$$Q(\{\lambda_\gamma\};\{A_\gamma\}) = Q(\{A_\gamma - \lambda_\gamma\};\{A_\gamma\}) \tag{22}$$

in terms of the macroscopic affinities driving the system out of equilibrium.

In the near-equilibrium regime, such a symmetry can be used to derive the Onsager reciprocity relations for transport coefficients [28] along with corresponding Green-Kubo formulas [29,30]. As this result is valid far from equilibrium, it also implies symmetry relations for the nonlinear response coefficients [4–6]. This theorem thus provides a unified framework to derive the linear and nonlinear response theory of nonequilibrium statistical mechanics.

The construction of this fluctuation theorem is based on the graph analysis of the master Eq. (1) introduced by Schnakenberg [26]. A graph is associated with the process in which the states σ are represented by vertices while the different edges correspond to the different mechanisms of transitions ρ between the states. In this scheme, the macroscopic affinities A_γ are identified by calculating the quantity (7) along the cycles of the graph. The current appearing in the fluctuation relation (19) then corresponds to the current crossing the edges used to close the cycles.

The Legendre transform $H(\{\alpha_\gamma\})$ of the generating function (20) is the decay rate of the probability that the currents take given values $\{\alpha_\gamma\}$

$$\mathrm{Prob}\left[\left\{\frac{1}{t}\int_0^t j_\gamma(t')dt' = \alpha_\gamma\right\}\right] \sim e^{-H(\{\alpha_\gamma\})t} \quad (t \to \infty). \tag{23}$$

The fluctuation theorem (22) translates into

$$H(\{-\alpha_\gamma\}) - H(\{\alpha_\gamma\}) = \sum_\gamma A_\gamma \alpha_\gamma. \tag{24}$$

If this relation is evaluated at the mean values $\alpha_\gamma = J_\gamma$ of the currents the decay rate vanishes $H(\{J_\gamma\})=0$ and we recover the entropy production

$$H(\{-J_\gamma\}) = \sum_\gamma A_\gamma J_\gamma = \left.\frac{d_{\mathrm{i}}S}{dt}\right|_{\mathrm{st}}. \tag{25}$$

From this viewpoint, Eq. (24) appears as a generalization of the fundamental Eq. (25) of nonequilibrium thermodynamics to fluctuating systems. By using Eqs. (11) and (21), Eq. (25) can be rewritten as

$$\left.\frac{d_{\mathrm{i}}S}{dt}\right|_{\mathrm{st}} = \frac{dq}{d\eta}(0) = \sum_\gamma A_\gamma \left.\frac{\partial Q}{\partial \lambda_\gamma}\right|_{\{\lambda_\epsilon=0\}}, \tag{26}$$

which shows that the fluctuation theorems for the dissipated work and the currents are closely related [5].

These results are applied in the following section to the model of molecular motor.

III. THE DISCRETE-STATE MODEL

A molecular motor is naturally functioning on a cycle of transformations between different mechanical and chemical states corresponding to different conformations of the protein complex. All these states form a cycle of periodicity L corresponding to the revolution by 360° for a rotary motor or the reinitialization for a linear motor. The transitions between the states $\{\mathrm{M}_\sigma\}$ are caused by the chemical reactions of the binding of the reactants $\mathrm{X}(\rho=+1)$ and the release of the products $\mathrm{Y}(\rho=+2)$

$$\mathrm{X} + \mathrm{M}_\sigma \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{M}_{\sigma+1} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} \mathrm{M}_{\sigma+2} + \mathrm{Y} \quad (\sigma = 1,3,\ldots,2L-1) \tag{27}$$

with a cyclic ordering $\mathrm{M}_{2L+1} \equiv \mathrm{M}_1$. The reversed reactions $(\rho=-1)$ and $(\rho=-2)$ are included to allow the system to reach a state of thermodynamic equilibrium if the nonequilibrium constraints are relaxed. The quantities k_ρ denote the reaction constants. For the F_1 rotary motor, the overall reaction is the hydrolysis of the reactant X=ATP into its products Y=ADP, $\mathrm{P_i}$ [16–20]. Viewed as motors, DNA and RNA polymerases are fuelled by the different triphosphates (ATP, CTP, GTP, and TTP or UTP) and the product is a double or single polymer strand.

For transmembrane motors such as $\mathrm{F_o}$ [15,17] or the bacterial flagellar motors [21], the reactant is $\mathrm{X}=\mathrm{H}^+$ on one side of the membrane and the product is $\mathrm{Y}=\mathrm{H}^+$ on the other side. We notice that sodium ions Na^+ play the role of protons H^+ in special $\mathrm{F_o}$ motors [31].

The probability to find the motor in the state M_σ is ruled by the master equation

$$\frac{dP(\sigma,t)}{dt} = w_{+2}P(\sigma-1,t) + w_{-1}P(\sigma+1,t) - (w_{+1}+w_{-2})P(\sigma,t), \quad \sigma \text{ odd}, \tag{28}$$

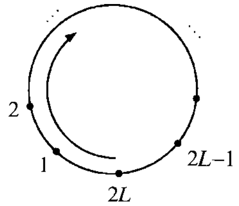

FIG. 1. Graph associated with the discrete-state model.

$$\frac{dP(\sigma,t)}{dt} = w_{+1}P(\sigma-1,t) + w_{-2}P(\sigma+1,t) - (w_{-1}+w_{+2})P(\sigma,t), \quad \sigma \text{ even}, \qquad (29)$$

with the transition rates

$$w_{+1} \equiv k_{+1}[\mathrm{X}],$$

$$w_{-1} \equiv k_{-1},$$

$$w_{+2} \equiv k_{+2},$$

$$w_{-2} \equiv k_{-2}[\mathrm{Y}]. \qquad (30)$$

The graph associated with the system is depicted in Fig. 1. It presents a unique cycle; hence a unique macroscopic affinity. As explained in the preceding section, the macroscopic affinity is obtained by calculating the quantity (7) along the cycle of the graph

$$A(\vec{C}) \equiv \ln \prod_{\sigma=1}^{2L} \frac{W(\sigma|\sigma+1)}{W(\sigma+1|\sigma)} = L \ln \frac{w_{+1}w_{+2}}{w_{-1}w_{-2}} = L \ln \frac{k_{+1}k_{+2}[\mathrm{X}]}{k_{-1}k_{-2}[\mathrm{Y}]}. \qquad (31)$$

It can also be expressed as $A=\Delta\mu/(k_{\mathrm{B}}T)$ in terms of the difference of chemical potentials $\Delta\mu \equiv \mu_{\mathrm{X}} - \mu_{\mathrm{Y}}$ of the chemical reaction (27).

The detailed balance conditions (5) should be satisfied at the thermodynamic equilibrium, which implies the vanishing of the affinity (31). Accordingly, equilibrium is reached if $w_{+1}w_{+2}=w_{-1}w_{-2}$ so that the reactant and product equilibrium concentrations must satisfy

$$\frac{[\mathrm{X}]_{\mathrm{eq}}}{[\mathrm{Y}]_{\mathrm{eq}}} = \frac{k_{-1}k_{-2}}{k_{+1}k_{+2}}. \qquad (32)$$

The stationary probability distribution is given by

$$P_{\mathrm{st}}(\sigma \text{ odd}) = \frac{w_{-1}+w_{+2}}{L(w_{+1}+w_{+2}+w_{-1}+w_{-2})}, \qquad (33)$$

$$P_{\mathrm{st}}(\sigma \text{ even}) = \frac{w_{+1}+w_{-2}}{L(w_{+1}+w_{+2}+w_{-1}+w_{-2})}. \qquad (34)$$

The steady state current (3) is constant according to Kirchhoff current law [26] and is given by

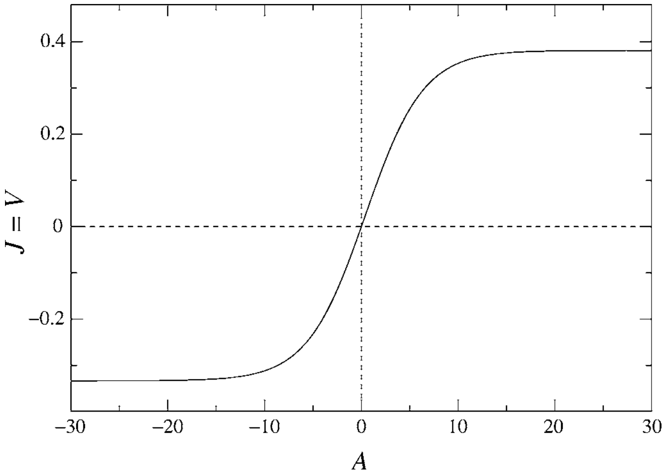

FIG. 2. Current $J=V$ versus the affinity A for the six-state model ($L=3$). The transition rates take the values $w_{+1}=2, w_{+2}=4, w_{-1}=1$ and w_{-2} is used as the dependent parameter.

$$J = \frac{w_{+1}w_{+2} - w_{-1}w_{-2}}{L(w_{+1}+w_{+2}+w_{-1}+w_{-2})} = \frac{w_{+1}w_{+2}(1-e^{-A/L})}{L(w_{+1}+w_{+2}+w_{-1}+w_{+1}w_{+2}e^{-A/L}/w_{-1})}. \qquad (35)$$

This corresponds to a kinetics of Michaelis-Menten type in the absence of the products Y of the reaction ($A=+\infty$) where the steady state current is given by

$$J = \frac{k_{+1}k_{+2}[\mathrm{X}]}{L(k_{+1}[\mathrm{X}]+k_{+2}+k_{-1})} = \frac{J_{\max}[\mathrm{X}]}{[\mathrm{X}]+K_{\mathrm{M}}} \qquad (36)$$

with the maximum value $J_{\max}=k_{+2}/L$ and the Michaelis-Menten constant $K_{\mathrm{M}}=(k_{+2}+k_{-1})/k_{+1}$. An example of dependence of the current on the affinity is depicted in Fig. 2.

A. The fluctuation theorem for the dissipated work

Let us first consider the fluctuation theorem for the dissipated work. Its generating function is given by the maximal eigenvalue of the operator (9) which here reads

$$w_{+2}^{\eta}w_{-2}^{1-\eta}g(\sigma-1) + w_{-1}^{\eta}w_{+1}^{1-\eta}g(\sigma+1) - (w_{+1}+w_{-2})g(\sigma) = -q(\eta)g(\sigma), \quad \sigma \text{ odd}, \qquad (37)$$

$$w_{+1}^{\eta}w_{-1}^{1-\eta}g(\sigma-1) + w_{-2}^{\eta}w_{+2}^{1-\eta}g(\sigma+1) - (w_{-1}+w_{+2})g(\sigma) = -q(\eta)g(\sigma), \quad \sigma \text{ even}. \qquad (38)$$

Its maximal eigenvector $\vec{g}_{\eta}$ is then given by

$$g(\sigma \text{ odd}) = 2(w_{-1}^{\eta}w_{+1}^{1-\eta} + w_{+2}^{\eta}w_{-2}^{1-\eta}) \qquad (39)$$

$$g(\sigma \text{ even}) = (r_1-r_2) + [(r_1-r_2)^2 + 4(w_{-1}^{\eta}w_{+1}^{1-\eta} + w_{+2}^{\eta}w_{-2}^{1-\eta}) \times (w_{+1}^{\eta}w_{-1}^{1-\eta} + w_{-2}^{\eta}w_{+2}^{1-\eta})]^{1/2}. \qquad (40)$$

The corresponding maximal eigenvalue is

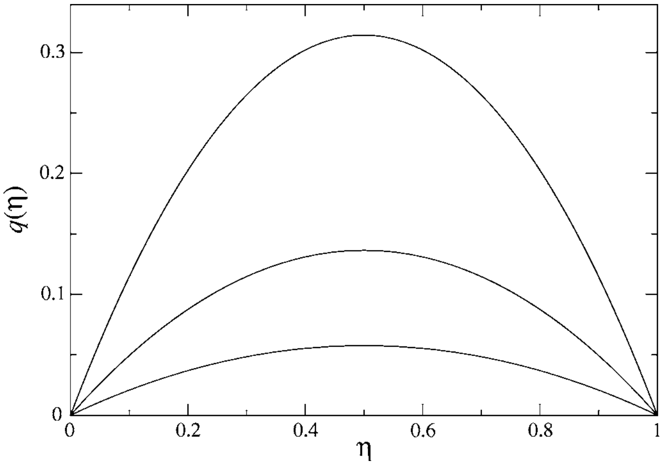

FIG. 3. Generating function (41) for the six-state model ($L=3$). The transition rates take the values $w_{+1}=2$, $w_{+2}=3$, $w_{-1}=1$, and $w_{-2}=3$, 2, 1.6. The different curves increase with the affinity.

$$q(\eta)=\frac{1}{2}\{w_{+1}+w_{+2}+w_{-1}+w_{-2}-[(w_{+1}+w_{-2}-w_{-1}-w_{+2})^2 + 4(w_{-1}^{\eta}w_{+1}^{1-\eta}+w_{+2}^{\eta}w_{-2}^{1-\eta})(w_{+1}^{\eta}w_{-1}^{1-\eta}+w_{-2}^{\eta}w_{+2}^{1-\eta})]^{1/2}\}, \tag{41}$$

which is depicted in Fig. 3 for different values of the affinity. It vanishes at equilibrium and satisfies the fluctuation theorem $q(\eta)=q(1-\eta)$. This function can be used to generate all the moments of the fluctuating quantity (7) and in particular the mean entropy production which is obtained by calculating its first derivative

$$\frac{dq}{d\eta}(0)=\frac{w_{+1}w_{+2}-w_{-1}w_{-2}}{w_{+1}+w_{+2}+w_{-1}+w_{-2}}\ln\frac{w_{+1}w_{+2}}{w_{-1}w_{-2}}. \tag{42}$$

This quantity is nothing else than the product of the mean current J (35) with the macroscopic affinity $A(\vec{C})$ (31)

$$\frac{d_iS}{dt}=JA, \tag{43}$$

which is the form expected from nonequilibrium thermodynamics [23].

It is interesting to note that the graph of Fig. 1 is identical to the one for a model of ion transport in membranes [6]. Indeed, one can check that in the case $k_{\pm\rho}=ke^{\pm\phi}$, $\rho=1,2$, we recover the solution of Ref. [6]. However, we here have two different types of transitions, which change the structure of the generating function by introducing the square and squareroot terms.

B. The fluctuation theorem for the rotation

We now consider the fluctuation theorem for the currents. In our case, we have seen in Sec. III that there is only one affinity and hence one current. The current fluctuation theorem thus takes the form

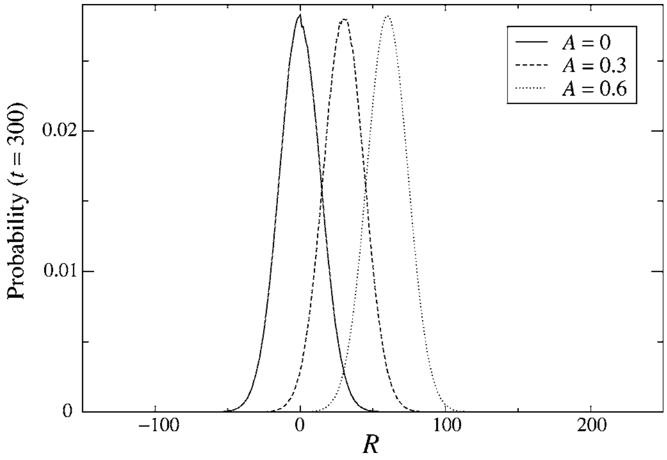

FIG. 4. Probability distribution functions of R_t for $t=300$ and for different values of the affinity in the six-state model ($L=3$). The transition rates take the values $k_{\pm 1}=10\ e^{\pm\phi}$, $k_{\pm 2}=15\ e^{\pm\phi}$ so that the affinity reads $A=12\phi$. ϕ takes the values 0, 0.025, and 0.05.

$$\frac{\mathrm{Prob}\left[\frac{1}{t}\int_0^t j(t')dt'=+\alpha\right]}{\mathrm{Prob}\left[\frac{1}{t}\int_0^t j(t')dt'=-\alpha\right]}\simeq e^{A(\vec{C})\alpha t}\quad (t\to\infty) \tag{44}$$

with $A(\vec{C})$ given by Eq. (31). As explained in Sec. II B, the current $j(t)$ is the current crossing the edge closing the corresponding cycle. In our case, there is a unique cycle and every edge could be chosen in order to close the cycle. Accordingly, the current appearing in Eq. (44) could be any of the $2L$ edges, meaning that the current fluctuation theorem is valid independently of our choice of the cross section used to measure the current.

The time integral of the current appearing in Eq. (44) here corresponds to the signed cumulated number of passages along one of the edges, which is equivalent to the number R_t of revolutions by 360° of the molecular motor during a time t

$$R_t=\int_0^t j(t')dt'. \tag{45}$$

This is an observable quantity and the fluctuation relation can be checked by numerical simulations. We used the Gillespie algorithm to simulate the master equation of the system. Since the system is ergodic, we may use a sufficiently long trajectory to verify the fluctuation theorem for the steady state in the $t\to\infty$ limit. The probability distribution of the current up to a time $t=300$ is depicted in Fig. 4 for different affinities. We see that the probability distribution functions are shifted to the right as we increase the affinity. The rotation velocity, i.e., the mean number of revolutions per unit time, is given by

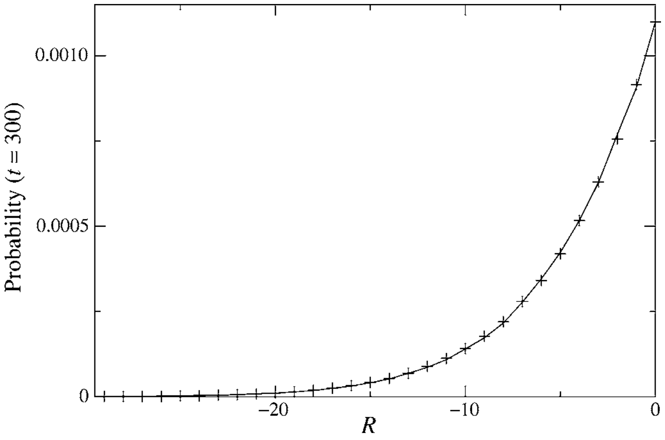

FIG. 5. Comparison between the prediction (47) of the fluctuation relation for negative events and the numerical simulations in the six-state model ($L=3$). The transition rates take the values $k_{\pm1}=10e^{\pm\phi}$, $k_{\pm2}=15\ e^{\pm\phi}$ with $\phi=0.03$ or $A=0.36$.

$$V = \lim_{t\to\infty}\frac{1}{t}\langle R_t\rangle = \frac{w_{+1}w_{+2}-w_{-1}w_{-2}}{L(w_{+1}+w_{+2}+w_{-1}+w_{-2})} = J, \qquad (46)$$

which corresponds to the mean current (35) in the nonequilibrium steady state. We also see in Fig. 4 that the probability to observe negative events decreases as the affinity is increased.

The fluctuation relation (44) is verified in Fig. 5: The probability of negative events is predicted to be given by

$$\mathrm{Prob}(R_t=-r) \simeq \mathrm{Prob}(R_t=r)e^{-rA}. \qquad (47)$$

The relation is clearly satisfied, even if the time t is finite. We notice that the negative events are already very rare.

Moreover, from the probability distribution functions of Fig. 4, it is possible to compute the generating function of the rotation

$$Q(\lambda;A) = \lim_{t\to\infty} -\frac{1}{t}\ln\langle e^{-\lambda R_t}\rangle \qquad (48)$$

by calculating the sum

$$e^{-tQ(\lambda)} \simeq \sum_{r=-\infty}^{+\infty} P(R_t=r)e^{-r\lambda}. \qquad (49)$$

The results are shown in Fig. 6 where they are compared with the function

$$Q(\lambda) = \frac{1}{2}\{w_{+1}+w_{+2}+w_{-1}+w_{-2}-[(w_{+1}+w_{-2}+w_{-1}+w_{+2})^2 + 4w_{+1}w_{+2}(e^{(\lambda-A)/L}+e^{-\lambda/L}-1-e^{-A/L})]^{1/2}\}, \qquad (50)$$

which is derived here below. This generating function has the symmetry $Q(\lambda)=Q(A-\lambda)$ of the fluctuation theorem. We point out that the fluctuations have a non-Gaussian character since a Gaussian distribution would have a quadratic generating function. Moreover, we see that the large deviations of the current and of the irreversible work (7) are of the same nature: We can recover the generating function (41) of the dissipated work $Z(t)$ by setting $\lambda=\eta A$: $q(\eta)=Q(A\eta)$.

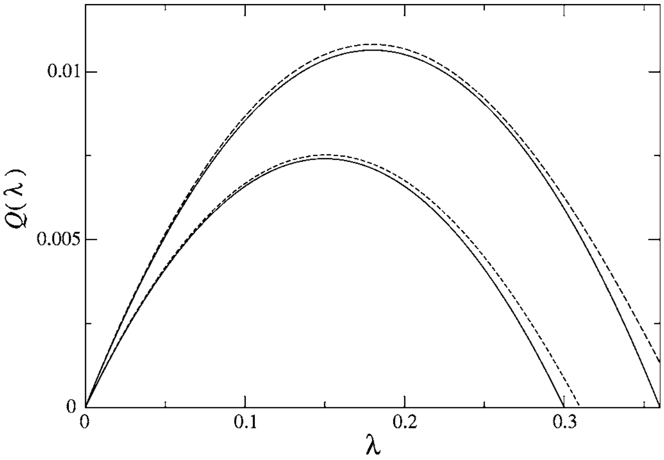

FIG. 6. Generating functions calculated with the direct statistical method in the six-state model (dashed lines). Comparison is made with the theoretical function (50). The transition rates take the same value as in the preceding figures and the affinity take the values $A=0.3$ and $A=0.36$.

This means that, from a large deviation point of view, the fluctuations of a trajectory between the complete revolutions of the motor are negligible so that the quantity $Z(t)$ can be assimilated to

$$Z(t) \equiv \ln\frac{W(\sigma_0|\sigma_1)W(\sigma_1|\sigma_2)\cdots W(\sigma_{n-1}|\sigma_n)}{W(\sigma_1|\sigma_0)W(\sigma_2|\sigma_1)\cdots W(\sigma_n|\sigma_{n-1})} \simeq AR_t. \qquad (51)$$

This result is exact for the mean value of $Z(t)$ [4,26] as can be seen from Eqs. (11) and (43), but we see that it also holds for the large fluctuations of $Z(t)$ in this particular example. The reason is that the rest term in Eq. (51) is bounded by some constants independent of t so that this term becomes negligible in the long-time limit. The discrepancy observed in Fig. 6 between the theoretical and numerical values of the generating function are due to the exponential decrease of the statistics of random events as $\lambda\to A$ where $Q(\lambda=A)=0$. In this case, the direct statistical method is not efficient to compute the generating function which require an exponentially growing statistics. This discrepancy is not caused by a finite-time effect [6] because it remains present if we increase the time while keeping constant the number of trajectories used in the statistics. Moreover, the very good agreement of the fluctuation relation seen in Fig. 5 is a good indication that the finite-time corrections are negligible.

The theoretical distribution (50) can be derived by the following reasoning. Let us consider the probability distribution $P(\sigma,r,t)$ to be in the state σ at time t while having done a displacement of r steps. The symmetry of the system imposes $P(1,r,t)=P(3,r,t)=\cdots=P(2L-1,r,t)$ and $P(2,r,t)=P(4,r,t)=\cdots=P(2L,r,t)$. The evolution equation is then given by

$$\frac{dP(1,r,t)}{dt} = [w_{+2}P(2,r-1,t)+w_{-1}P(2,r+1,t)] - (w_{+1}+w_{-2})P(1,r,t), \qquad (52)$$

$$\frac{dP(2,r,t)}{dt} = [w_{+1}P(1,r-1,t) + w_{-2}P(1,r+1,t)] - (w_{+2}+w_{-1})P(2,r,t). \qquad (53)$$

Now introducing the generating functions

$$F(\xi,t) = L\sum_{r=-\infty}^{+\infty} e^{-r\xi}P(1,r,t), \qquad (54)$$

$$G(\xi,t) = L\sum_{r=-\infty}^{+\infty} e^{-r\xi}P(2,r,t), \qquad (55)$$

the system becomes

$$\frac{\partial F(\xi,t)}{\partial t} = [w_{+2}e^{-\xi} + w_{-1}e^{\xi}]G(\xi,t) - (w_{+1}+w_{-2})F(\xi,t),$$

$$\frac{\partial G(\xi,t)}{\partial t} = [w_{+1}e^{-\xi} + w_{-2}e^{\xi}]F(\xi,t) - (w_{+2}+w_{-1})G(\xi,t), \qquad (56)$$

with the initial conditions

$$F(\xi,t=0) = L\sum_{r=-\infty}^{+\infty} e^{-r\xi}\delta_{r0}P_{\rm st}(1) = LP_{\rm st}(1), \qquad (57)$$

$$G(\xi,t=0) = L\sum_{r=-\infty}^{+\infty} e^{-r\xi}\delta_{r0}P_{\rm st}(2) = LP_{\rm st}(2), \qquad (58)$$

corresponding to the stationary state. The solution of the system (56) is given by the exponential of the time evolution matrix $\hat{M}_\xi$ so that

$$F(\xi,t) = L\exp\left(\frac{a+d}{2}t\right)\left\{\left[\cosh\left(\frac{\Delta t}{2}\right) + \frac{a-d}{\Delta}\sinh\left(\frac{\Delta t}{2}\right)\right]P_{\rm st}(1) + \frac{2b}{\Delta}\sinh\left(\frac{\Delta t}{2}\right)P_{\rm st}(2)\right\}, \qquad (59)$$

$$G(\xi,t) = L\exp\left(\frac{a+d}{2}t\right)\left\{\frac{2c}{\Delta}\sinh\left(\frac{\Delta t}{2}\right)P_{\rm st}(1) + \left[\cosh\left(\frac{\Delta t}{2}\right) + \frac{d-a}{\Delta}\sinh\left(\frac{\Delta t}{2}\right)\right]P_{\rm st}(2)\right\}, \qquad (60)$$

where $a=M_{11}$, $b=M_{12}$, $c=M_{21}$, $d=M_{22}$, and $\Delta=\sqrt{(a-d)^2+4bc}$. If we are only interested in the total displacement regardless of the final position of the motor, we have to look at the quantity $F(\xi,t)+G(\xi,t)=\langle \mathrm{e}^{-\xi S_t}\rangle$, where S_t is the signed number of steps the motor performs during a time t. This corresponds to the finite time generating function of the displacement as $e^{-t\tilde{Q}(\xi,t)}=\langle e^{-\xi S_t}\rangle$. The long time behavior is controlled by the maximal eigenvalue $\hat{M}_\xi\vec{f}_\xi=-\tilde{Q}(\xi)\vec{f}_\xi$

$$\tilde{Q}(\xi) = \frac{1}{2}\{w_{+1}+w_{+2}+w_{-1}+w_{-2} - [(w_{+1}+w_{-2}+w_{-1}+w_{+2})^2 + 4w_{+1}w_{+2}(e^{2\xi-A/L}+e^{-2\xi}-1-e^{-A/L})]^{1/2}\}, \qquad (61)$$

which gives the infinite time generating function of the displacement of the motor

$$\tilde{Q}(\xi) = \lim_{t\to\infty} -\frac{1}{t}\ln\langle e^{-\xi S_t}\rangle \qquad (62)$$

as can be checked from the solution (60). This generating function presents the symmetry

$$\tilde{Q}(\xi) = \tilde{Q}\left(\frac{A}{2L}-\xi\right). \qquad (63)$$

The finite time corrections to the fluctuation theorem (63) can be calculated from the solution (60). The corresponding current and higher-order moments of the distribution can be derived in a systematic way from this generating function. The displacement of the motor will thus satisfy the fluctuation theorem

$$\frac{\mathrm{Prob}(S_t/t=+\gamma)}{\mathrm{Prob}(S_t/t=-\gamma)} \simeq \exp\frac{A\gamma t}{2L} \quad (t\to\infty). \qquad (64)$$

During a random trajectory over a time interval t, the total displacement S_t can be written as $S_t=2LR_t+\delta_t$, where R_t is the number of revolutions and δ_t can only take integer values between 0 and $2L-1$ depending on the stochastic trajectory. This term is necessary because a random trajectory does not necessarily consist in an integer number of revolutions. Since this term is bounded, each revolution roughly corresponds to $2L$ steps we can guess that the scaling $\lambda\equiv 2L\xi$ must be made to relate the generating function (61) to Eq. (50): $Q(\lambda)=\tilde{Q}(\lambda/2L)$. In the long-time limit, the quantities R_t/t and S_t/t thus have the same fluctuations so that the rotation of the motor satisfies the large-deviation relation

$$\frac{\mathrm{Prob}(R_t/t=+\alpha)}{\mathrm{Prob}(R_t/t=-\alpha)} \simeq \exp A\alpha t \ (t\to\infty), \qquad (65)$$

which is in very good agreement with the numerical results.

The generating function (50) allows us to derive not only the mean current but also the higher-order moments by differentiation. Indeed

$$\left.\frac{\partial Q}{\partial\lambda}\right|_{\lambda=0} = \lim_{t\to\infty}\frac{\langle R_t\rangle}{t} = V = \frac{w_{+1}w_{+2}-w_{-1}w_{-2}}{L(w_{+1}+w_{+2}+w_{-1}+w_{-2})}, \qquad (66)$$

which is the same as Eq. (35). Continuing the differentiation, we get the diffusion coefficient of rotation as

$$\left.\frac{\partial^2 Q}{\partial\lambda^2}\right|_{\lambda=0} = \lim_{t\to\infty} -\frac{\langle (R_t-\langle R_t\rangle)^2\rangle}{t} = -2D, \qquad (67)$$

which is explicitly given by

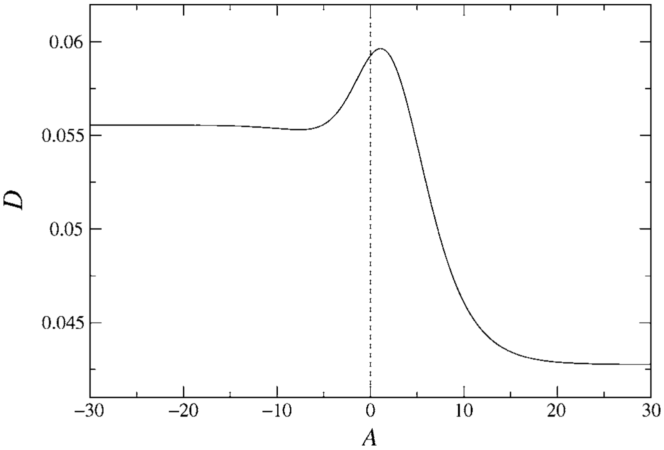

FIG. 7. Diffusion coefficient of the rotation as a function of the affinity in the six-state model ($L=3$). The transition rates take the values $w_{+1}=2$, $w_{+2}=4$, $w_{-1}=1$, and w_{-2} is used as the dependent parameter.

$$D = \frac{w_{+1}w_{+2} + w_{-1}w_{-2}}{2L^2(w_{+1} + w_{+2} + w_{-1} + w_{-2})} - \frac{(w_{+1}w_{+2} - w_{-1}w_{-2})^2}{L^2(w_{+1} + w_{+2} + w_{-1} + w_{-2})^3}. \tag{68}$$

A typical dependence of the diffusion coefficient is depicted in Fig. 7. The diffusion coefficient characterizes the fluctuations in the rotation of the motor. The correlation time of the successive revolutions can be defined as the decay time of the time correlation function of the random variable $\cos(2\pi R_t)$ for instance. The correlation time is related to the diffusion coefficient by $\tau=1/[(2\pi)^2 D]$. On the other hand, the mean period of one revolution is given by $\mathcal{T}=1/|J|$. The persistence of rotation can be characterized by the quality factor $\mathcal{Q}=2\pi\tau/\mathcal{T}=|J|/(2\pi D)$. The rotation is systematic if $\mathcal{Q}>1$. This quality factor vanishes around the thermodynamic equilibrium where the fluctuations are important and preclude the possibility of persistent rotation. For the present model, the maximum value of the quality factor is $\mathcal{Q}_{\max}=4L$ in which case rotation is not much affected by the fluctuations. We notice that the verification of the fluctuation theorem by a direct statistics is easier in the regime where the quality factor is $\mathcal{Q}<1$. On the other hand, the cumulants derived from the generating function (50) are related to those derived for the displacement of the motor by

$$\left.\frac{\partial^n \tilde{Q}}{\partial \xi^n}\right|_{\xi=0} = (2L)^n \left.\frac{\partial^n Q}{\partial \lambda^n}\right|_{\lambda=0}. \tag{69}$$

This means in particular that the fluctuation theorem (63) or (64) for the displacement of the motor should be easier to observe experimentally than the one (65) for the full revolutions.

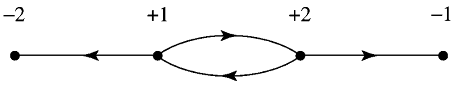

FIG. 8. The states +1 and +2 correspond to the reactions driving the motor in a forward rotation. The states −2 and −1 are the absorbing states and are reached by a backward transition.

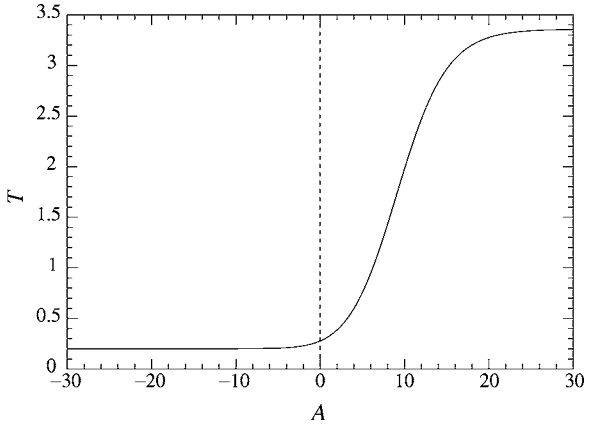

FIG. 9. Mean waiting time T before a negative fluctuation in the six-state model ($L=3$). The transition rates take the values $w_{+1}=2$, $w_{+2}=4$, $w_{-1}=1$, and w_{-2} is used as the dependent parameter.

Another consequence of the current fluctuation theorem concerns the consumption of molecules X of the chemical fuel. Indeed, for every revolution of the motor, L molecules of X are consumed. During a random trajectory over a time interval t, the number N_t of molecules X consumed can be written as $N_t=LR_t+\epsilon_t$ where ϵ_t depends on the stochastic trajectory and can only take the values $0,1,2,\ldots,L-1$. This term is necessary because the number N_t does not necessarily consist in an integer number of revolutions. Since this term is bounded, the quantities R_t/t and N_t/t have the same fluctuations in the long-time limit, whereupon the consumption of X should satisfy the large-deviation relation

$$\frac{\mathrm{Prob}(N_t=+n)}{\mathrm{Prob}(N_t=-n)} \simeq \exp\frac{An}{L} \quad (t\to\infty). \tag{70}$$

Finally, we notice that the current fluctuation theorem can be used to obtain the affinity of the process from the probability distribution of the current, regardless of the detailed mechanisms of transitions between the states (which are usually unknown). This probability distribution function can also be used to calculate the mean current so that we can estimate the entropy production of the process thanks to the formula (43). With the help of the current fluctuation theorem, the entropy production can thus be obtained from the sole knowledge of the current distribution function, without any knowledge of the microscopic transition mechanisms.

C. Mean time before negative fluctuations

We have shown in the preceding section that the probability to observe negative fluctuations decreases exponentially with the affinity driving the system out of equilibrium. Since molecular motors usually operate far from equilibrium, one can thus expect that negative fluctuations could be rare to observe. Our purpose in this section is to derive the probability distribution function of the time necessary to observe a negative step of the motor.

This can be treated as a first-time passage problem: The motor follows a trajectory but is absorbed as soon as it makes a backward step. Using the symmetry of the motor, we can reduce the problem to the following system. The motor can jump between the states +1 and +2 corresponding to the reactions $\rho=+1$ and $\rho=+2$. The motor can also jump backward because of the reactions $\rho=-1$ and $\rho=-2$, which are associated with some states -1 and -2. As these states are reached with a backward transition, they correspond to the absorbing states of the system and cannot be left. This system is schematically represented in Fig. 8.

The absorbing boundary conditions are taken into account by imposing

$$P(-1,t) = P(-2,t) = 0 \tag{71}$$

so that the probability distribution obeys the evolution equation

$$\frac{dP(+1,t)}{dt} = w_{+2}P(+2,t) - (w_{+1} + w_{-2})P(+1,t),$$

$$\frac{dP(+2,t)}{dt} = w_{+1}P(+1,t) - (w_{+2} + w_{-1})P(+2,t). \tag{72}$$

Therefore, the evolution matrix $\hat{M}$ of the system (72) does not conserve the total probability $\bar{P}(t) \equiv P(+1,t)+P(+2,t)$ which typically decreases exponentially in time. The probability distribution function of the absorbing time is thus given by

$$f(t) = -\frac{d\bar{P}(t)}{dt}. \tag{73}$$

In particular, the mean absorption time is given by

$$T = \int_0^\infty dt\ t\ f(t) = \int_0^\infty dt\ \bar{P}(t) \tag{74}$$

after an integration by parts. The solution of the system (72) can be expressed as

$$\vec{P}(t) = e^{\hat{M}t}\vec{P}(0) \tag{75}$$

so that the mean absorbing time becomes

$$T = \int_0^\infty dt \bar{P}(t) = \int_0^\infty dt \sum_k [e^{\hat{M}t}\vec{P}(0)]_k$$

$$= \int_0^\infty dt \sum_{k,l} [e^{\hat{M}t}]_{kl}[\vec{P}(0)]_l = \sum_{k,l} (-\hat{M}^{-1})_{kl}[\vec{P}(0)]_l. \tag{76}$$

Calculating the inverse matrix $\hat{M}^{-1}$

$$\hat{M}^{-1} = -\frac{1}{\det \hat{M}} \begin{pmatrix} w_{+2} + w_{-1} & w_{+2} \\ w_{+1} & w_{+1} + w_{-2} \end{pmatrix}, \tag{77}$$

where $\det \hat{M} = (w_{+1}+w_{-2})(w_{+2}+w_{-1}) - w_{+1}w_{+2}$, and using the initial conditions $P(+i,0) = LP_{\rm st}(i)$ corresponding to the stationary state, one finds that the mean time before observing a backward step is given by

$$T = \frac{(w_{-1} + w_{+2})(w_{+1} + w_{+2} + w_{-2}) + (w_{-2} + w_{+1})(w_{+1} + w_{+2} + w_{-1})}{(w_{+1} + w_{+2} + w_{-1} + w_{-2})(w_{+1}w_{-1} + w_{+2}w_{-2} + w_{-1}w_{-2})}. \tag{78}$$

In the limit case where $w_{+1}, w_{+2} \gg w_{-1}, w_{-2}$, the mean waiting time becomes

$$T \simeq \frac{w_{+1} + w_{+2}}{w_{+1}w_{-1} + w_{+2}w_{-2}}. \tag{79}$$

This mean waiting time is depicted in Fig. 9 for the same values of the transition rates as in Figs. 2 and 7.

Similarly, one can obtain the probability distribution and all its moments $\langle t^n \rangle$ because the exponential of the evolution matrix $\hat{M}$ can be calculated exactly.

Moreover, one can evaluate the probability that a trajectory will ever reach a total displacement of -1 step. This gives the approximate fraction of the trajectories required in order to be able to observe the negative events considered in the fluctuation relation (64). This is different from the previous consideration where we have been interested in the negative fluctuations regardless of the total displacement. This probability can be obtained by considering a random walk where the sites correspond to the total displacement made by the motor. The initial condition corresponds to a null displacement, and we must consider the two cases where the motor starts from a site of type $\sigma=1$ or from a site $\sigma=2$, weighted with their respective probabilities. A well-known result in the theory of Markovian random walks (see for instance Ref. [32]) gives the probability which is given by

$$\mathcal{P} = \frac{w_{-1} + w_{+2}}{w_{+1} + w_{+2} + w_{-1} + w_{-2}} \frac{F(w_{-2}/w_{+1})}{1 + F(w_{-2}/w_{+1})} + \frac{w_{+1} + w_{-2}}{w_{+1} + w_{+2} + w_{-1} + w_{-2}} \frac{F(w_{-1}/w_{+2})}{1 + F(w_{-1}/w_{+2})}, \tag{80}$$

where

$$F(x) = \frac{x + e^{-A/L}}{1 - e^{-A/L}} \tag{81}$$

if $A > 0$. For $A \leq 0$, this probability is equal to unity $\mathcal{P}=1$, because the mean motion is backward. This probability is depicted in Fig. 10 for the same values of the transition rates as in Figs. 2 and 7. These results suggest that the fluctuation

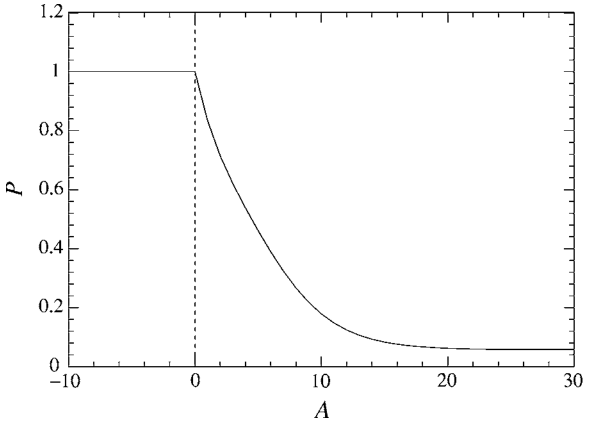

FIG. 10. Probability $\mathcal{P}$ that a trajectory ever reaches a total displacement of −1 step in the six-state model ($L=3$). The transition rates take the values $w_{+1}=2$, $w_{+2}=4$, $w_{-1}=1$, and w_{-2} is used as the dependent parameter.

theorem becomes more and more evident if the system is close to equilibrium and that the random backward rotations of the motor become rare away from the equilibrium.

IV. THE F_1 MOLECULAR MOTOR

In this section, we apply the discrete-state model described here above to the F_1 motor studied by Yasuda and coworkers in Ref. [19]. The F_1 protein complex is composed of three large α and β subunits circularly arranged around a smaller γ subunit. The three β subunits are the reactives sites for the hydrolysis of ATP, while the γ subunit plays the role of rotation shaft to which a bead of 40 nm diameter is glued. The mechanism of rotational catalysis was proposed by Boyer using a bisite activation [20]. Nevertheless, experimental data cannot distinguish for the moment between the bisite and three-site activations. The observation [19] clearly shows that the rotation takes place in six steps: ATP binding induces a rotation of about 90° followed by the release of ADP and P_i with a rotation of about 30°. Therefore, the hydrolysis of one ATP corresponds to a rotation by 120° and a revolution of 360° to three sequential ATP hydrolysis in the three β subunits. The six successive states of the hydrolytic motor $M=F_1$ can thus be specified by the angle θ of the shaft and the occupancy of the sites of the three β subunits as

$$M_1 = M[\theta=0,(ADP+P_i,\ \varnothing\ ,x)],$$

$$M_2 = M\left[\theta=\frac{\pi}{2},(ADP+P_i,ATP,x)\right],$$

$$M_3 = M\left[\theta=\frac{2\pi}{3},(\varnothing,ADP+P_i,\ x)\right],$$

$$M_4 = M\left[\theta=\frac{5\pi}{6},(x,ADP+P_i,ATP)\right],$$

$$M_5 = M\left[\theta=\frac{4\pi}{3},(x,\ \varnothing\ ,ADP+P_i)\right], \qquad (82)$$

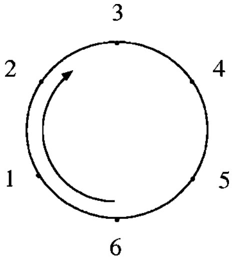

FIG. 11. Graph associated with the six-state model.

$$M_6 = M\left[\theta=\frac{11\pi}{6},(ATP,x,ADP+P_i)\right],$$

where x stands either for $\varnothing$ or ADP for the bi- or three-site mechanism. If the site is empty, the F_1 complex jumps to the following state with the rate $k_{+1}[ATP]$, and with the rate k_{+2} if the site is occupied. The backward transitions being possible, the complex can jump to the preceding state with the rate k_{-1} if the site is occupied and the rate $k_{-2}[ADP][P_i]$ if it is empty. This process can be summarized by the following reaction scheme:

$$ATP + M_\sigma \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} M_{\sigma+1} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} M_{\sigma+2} + ADP + P_i (\sigma = 1,3,5) \qquad (83)$$

with a cyclic ordering $M_7 \equiv M_1$. This is the model (27) with $L=3$ with the transition rates

$$w_{+1} \equiv k_{+1}[ATP],$$

$$w_{-1} \equiv k_{-1},$$

$$w_{+2} \equiv k_{+2},$$

$$w_{-2} \equiv k_{-2}[ADP][P_i]. \qquad (84)$$

The graph of this six-state model is depicted in Fig. 11. The threefold symmetry of the F_1-ATPase is taken into account in the model by the symmetry of the transition rates.

The standard free enthalpy of hydrolysis is equal to

$$\Delta G^0 = G^0_{ATP} - G^0_{ADP} - G^0_{P_i} = 50 \text{ pN nm}. \qquad (85)$$

The temperature of the experiment of Ref. [19] is 23°C so that the equilibrium concentrations of the reactant and products obey

$$\frac{[ATP]_{eq}}{[ADP]_{eq}[P_i]_{eq}} = \frac{k_{-1}k_{-2}}{k_{+1}k_{+2}} = e^{-\Delta G^0/k_B T} = 4.89 \cdot 10^{-6} \text{ M}^{-1}, \qquad (86)$$

which is a constraint on the reaction constants from equilibrium thermodynamics. We notice that, under physiological conditions, the concentrations are about $[ATP] \simeq 10^{-3}$ M, $[ADP] \simeq 10^{-4}$ M, and $[P_i] \simeq 10^{-3}$ M, so that ATP is in large excess with respect to its equilibrium concentration $[ATP]_{eq} \simeq 4.89 \cdot 10^{-13}$ M, which shows that the system is typically very far from equilibrium.

The reaction constants $k_{\pm\rho}$ can be determined from the experimental data [22]. In the absence of the products, the

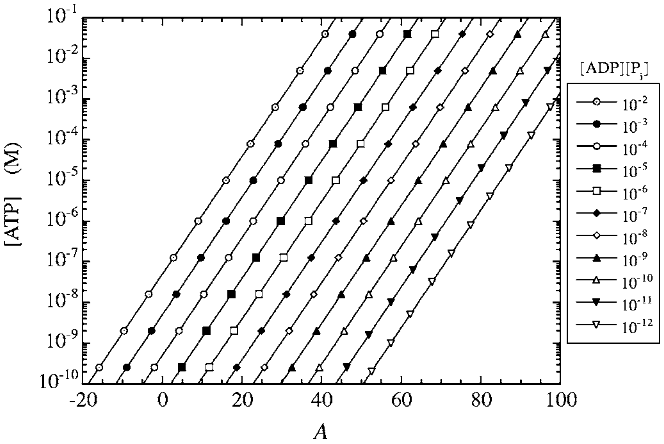

FIG. 12. Concentration of ATP versus the affinity A according to Eq. (89).

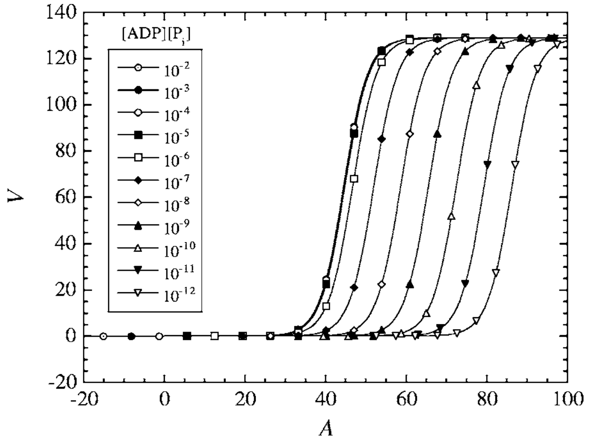

FIG. 13. Mean rotation velocity V of the F_1 motor with a bead of 40 nm diameter versus the affinity A for different concentrations $[\mathrm{ADP}][\mathrm{P_i}]$ of the products.

rotation velocity is observed to follow a Michaelis-Menten kinetics in agreement with Eq. (36) of the model

$$V = \frac{V_{\max}[\mathrm{ATP}]}{[\mathrm{ATP}] + K_{\mathrm{M}}} \tag{87}$$

with the maximum velocity $V_{\max}=k_{+2}/3=129\pm 9$ revolutions per second and the Michaelis-Menten constant $K_{\mathrm{M}}=(k_{+2}+k_{-1})/k_{+1}=15\pm 2\ \mu\mathrm{M}$. Furthermore, the dependence of the rotation velocity on the product concentrations can be used to obtain that $k_{-2}/k_{+1}=13.7\pm 0.4\ \mathrm{M}^{-1}$ [22]. Thermodynamic equilibrium (86) provides the last relation so that the reaction constants are thus given by

$$k_{+1} = (2.6 \pm 0.5)10^{7}\ \mathrm{M}^{-1}\ \mathrm{s}^{-1},$$

$$k_{-1} = (138 \pm 34)10^{-6}\ \mathrm{s}^{-1},$$

$$k_{+2} = (387 \pm 27)\ \mathrm{s}^{-1},$$

$$k_{-2} = (3.5 \pm 0.8)10^{8}\ \mathrm{M}^{-2}\ \mathrm{s}^{-1}. \tag{88}$$

We observe that these reaction constants range over about 12 orders of magnitude, which is characteristic of a stiff stochastic process.

The affinity of the cycle of Fig. 11 is defined as

$$A \equiv 3 \ln \frac{k_{+1}k_{+2}[\mathrm{ATP}]}{k_{-1}k_{-2}[\mathrm{ADP}][\mathrm{P_i}]}, \tag{89}$$

which vanishes at equilibrium. Figure 12 shows how the concentration of ATP varies with the affinity for different concentrations of the products. We see that the ATP concentration is always very small at equilibrium.

The mean rotation velocity is depicted in Fig. 13 as a function of the affinity (89) for different concentrations of the products. We observe that the V-A curve is highly nonlinear as a consequence of the stiffness of the process. Even the vanishing of the mean velocity at the thermodynamic equilibrium $A=0$ is not visible in Fig. 13. A zoom is carried out in the vicinity of equilibrium in Fig. 14 where we observe that indeed the mean velocity vanishes linearly with the affinity as expected. This linear regime does not extend by more than one decade around the equilibrium concentration. Typically, the motor is very far from equilibrium and is functioning in the nonlinear regime. This shows the crucial importance of these nonlinear regimes of nonequilibrium thermodynamics for the understanding of biological molecular motors.

Another consequence of the stiffness of the motor is that the diffusion coefficient depicted in Fig. 15 is small relative to the mean velocity. For most values of the affinity, the ratio of the mean velocity to the diffusion coefficient is about $V/D \simeq 6$, which is characteristic of a correlated rotation slightly affected by the fluctuations.

Nevertheless, the fluctuation theorem holds even far from equilibrium as we can see in Fig. 16 which depicts the generating function (41) of the dissipated work. We observe that, indeed, the symmetry (10) of the fluctuation theorem is well satisfied for different concentrations of ATP. Moreover, the fluctuation theorem can be directly verified from the statistics of the random steps forward and backward as shown in Fig. 17 where we show that the fluctuation relation

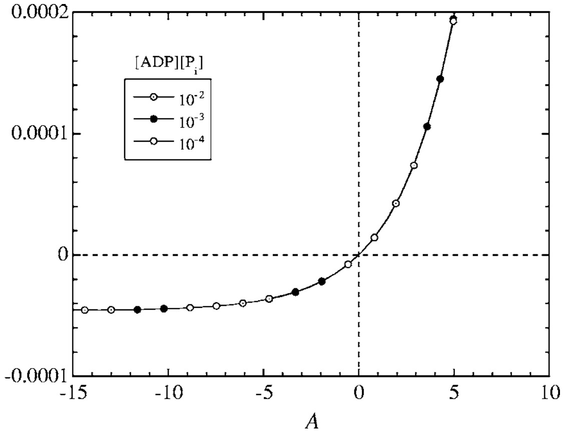

FIG. 14. Zoom of Fig. 13 giving the mean rotation velocity V of the F_1 motor versus the affinity A around the equilibrium at $A=0$ for different concentrations $[\mathrm{ADP}][\mathrm{P_i}]$ of the products.

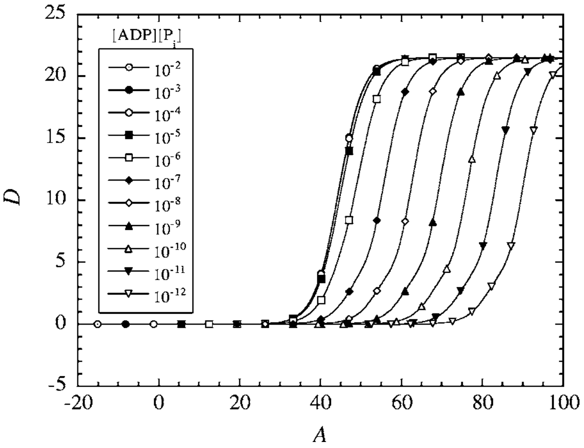

FIG. 15. Diffusion coefficient D of the F_1 motor with a bead of 40 nm diameter versus the affinity A for different concentrations $[\mathrm{ADP}][\mathrm{P_i}]$ of the products.

$$\mathrm{Prob}(S_t = s) \simeq \mathrm{Prob}(S_t = -s)e^{-sA/6} \tag{90}$$

for the probability $\mathrm{Prob}(S_t = s)$ for $s = S_t$ steps over a time interval t is indeed satisfied. This verification requires a statistics proportional to the inverse of the probability given by Eq. (80) which is given in the caption of Fig. 17. As seen in Fig. 17, the probability distribution of the displacement takes here a specific form where the odd displacements are almost never occurring. Indeed, for these values of the concentrations of the chemical species, the probability to be on odd sites is about four orders of magnitude lower than the probability to be on even sites. The system almost never stays on odd sites and immediately jumps to the next or previous sites.

As discussed previously, the F_1 molecular motor is a stiff process and typically operates in the nonlinear regime with a quality factor close to unity. This means that large fluctuations with negative events rapidly become inobservable. Nevertheless, from the exact solution (60) one can see that the finite time corrections to the fluctuation theorem usually quickly become negligible. Therefore, one can hope to observe the symmetry relation (63) even for systems further away from equilibrium if combined with a small enough observation time. In cases where the finite time corrections are not negligible, one can still calculate the finite time generating function from experimental data and compare to the exact solution (60) at finite time.

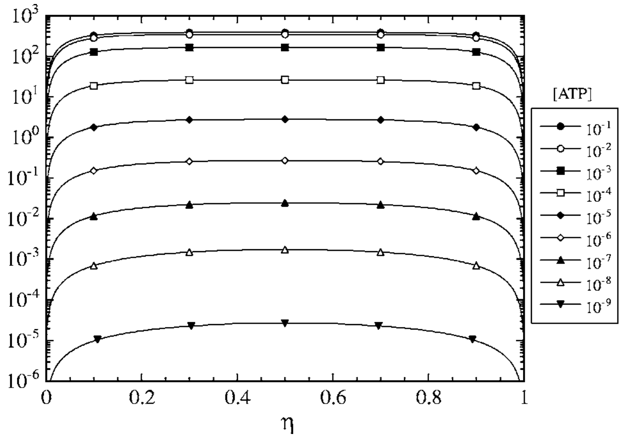

FIG. 16. Generating function $q(\eta)$ of the dissipated work of the F_1 motor with a bead of 40 nm diameter versus the parameter η for different concentrations of [ATP] and the fixed value $[\mathrm{ADP}][\mathrm{P_i}]=10^{-4}$ M^2. We notice that $q(\eta)=0$ at equilibrium where $[\mathrm{ATP}]_{\mathrm{eq}}=4.89\cdot 10^{-10}$ M.

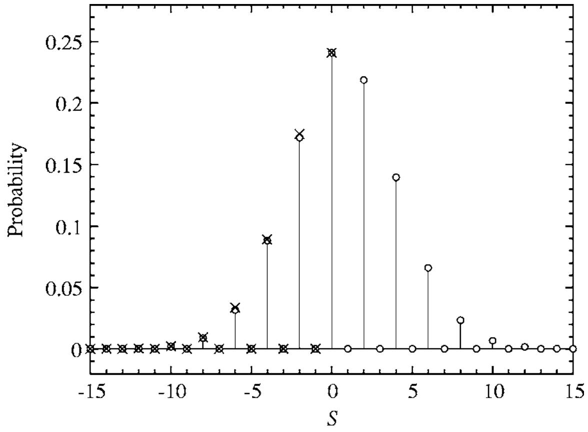

FIG. 17. Probability $\mathrm{Prob}(S_t=s)$ (open circles) that the F_1 motor performs $s=S_t$ steps during the time interval $t=10^4$ s compared with the expression $\mathrm{Prob}(S_t=-s)e^{-sA/6}$ (crosses) expected from the fluctuation theorem for $[\mathrm{ATP}]=6\cdot 10^{-8}$ M and $[\mathrm{ADP}][\mathrm{P_i}]=10^{-2}$ M^2. The probability (80) is here equal to $\mathcal{P}=0.8$.

According to Eq. (18), the maximum work which can be done per revolution by the F_1 motor is $\Delta G=3(\mu_{\mathrm{ATP}}-\mu_{\mathrm{ADP}}-\mu_{\mathrm{P_i}})=Ak_{\mathrm{B}}T$ which can be read in Fig. 12 with $k_{\mathrm{B}}T=4.1$ pN nm$=4.1\cdot 10^{-11}$ J.

V. CONCLUSIONS

Molecular motors are functioning at the nanoscale where the fluctuations are important in particular in the chemical reactions maintaining these nanosystems out of equilibrium. Accordingly, they require a stochastic description to take into account the randomness of the reactive events and of the environment. In this description, a central quantity of interest is the affinity or thermodynamic force, which is given in terms of the free enthalpy of the chemical reactions. The affinity thus plays a crucial role in the nonequilibrium thermodynamics of molecular motors.

In the present paper, we have shown that the affinity can be determined thanks to large-deviation relationships known under the name of fluctuation theorems, which we have here applied to molecular motors. The fluctuation theorems are connected to Jarzynski nonequilibrium work theorem, as discussed in Sec. II. These theorems express a fundamental symmetry of the molecular fluctuations, which has its origin in the microreversibility. This symmetry concerns different quantities such as the work dissipated in the irreversible processes, the currents across the system, as well as the displacement for linear motors or the rotation for rotary motors.

Considering a discrete-state stochastic model of molecular motors, we have obtained fluctuation theorems for these related quantities. The probabilities of their fluctuations obey general relationships which are valid far from thermodynamic equilibrium and which say that the probabilities of the forward to backward random motions of the motor are in a ratio which only depends on the affinity of the process and the number of reactive steps which have occurred during some time interval. This provides a method to measure experimentally the affinity of the nonequilibrium process driving the molecular motor. The fluctuation theorem can be expressed for the number of revolutions of a rotary motor as well as for the number of steps or the work dissipated during some time interval. These quantities are related to each other by a proportionality factor. The theory also provides the mean current or mean rotation velocity as a function of the affinity, as well as the diffusion coefficient characterizing the fluctuations around the mean motion.

We have also studied the time required to observe steps in the direction opposite to the mean motion. The shorter this time, the higher the statistics of the backward random events needed to use the fluctuation theorem. We have in particular shown that this time is shorter if the fluctuating quantity is the number of steps instead of the number of revolutions.

We have applied these considerations to the F_1 motor, which has been experimentally investigated by Yasuda and coworkers [19]. This molecular motor is a protein complex for the synthesis of ATP in mitochondria. In vitro, a bead can be glued to its shaft and its rotation can be observed under nonequilibrium conditions fixed by the concentration of ATP with respect to the concentrations of the products of ATP hydrolysis. The F_1 motor is thus an example of a nonequilibrium nanosystem affected by molecular fluctuations. The reaction constants of the discrete-state stochastic model can be fitted to the experimental data, which reveals that the process is stiff because the reaction constants range over 12 orders of magnitude. Accordingly, the response of the system to the nonequilibrium constraints, i.e., the mean rotation velocity versus the affinity, is a highly nonlinear function. The linear regime only extends over a very small interval of concentrations around chemical equilibrium. Under typical physiological conditions as well as in the experiments by Yasuda and coworkers [19], the F_1 motor functions very far from equilibrium, deep in the nonlinear regime of nonequilibrium thermodynamics. This nonlinearity confers to the rotational motion a robustness which does not exist near equilibrium. This robustness can be characterized by the quality factor of the motor, which is given in terms of the ratio of the mean rotation velocity over the diffusion coefficient. In the nonlinear regime, the quality factor reaches a value close to unity meaning that the successive rotations are statistically correlated and thus remain essentially unaffected by the molecular fluctuations. Nevertheless, we can show that the fluctuation theorem is satisfied close and far from equilibrium, in both the linear and nonlinear regimes. The fluctuation theorem here says that the ratio of the probability of a forward rotation of the shaft to the probability of a backward rotation determines the affinity of the process. This provides a method to measure experimentally this affinity which is the free enthalpy of the chemical reaction of hydrolysis. The fluctuation theorem can therefore be used to obtain key information on the nonequilibrium thermodynamics of molecular motors.

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