Fluctuation theorem, 
nonequilibrium work, and molecular machines

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A brief review is presented of recent advances in nonequilibrium statistical thermodynamics, leading to the so-called fluctuation theorems and nonequilibrium work relations. These new results concern the forward and reversed fluctuations of molecular machines driven out of equilibrium by energy sources, as illustrated with the F$_{1}$-ATPase rotary motor.

I. INTRODUCTION

We could paraphrase Professor Noyori [1] and say that the science of molecular machines is four-dimensional chemistry since their 3D structure ($x,y,z$) is coupled to their motion and kinetics ($t$). In condensed phases at equilibrium, the motion is erratic with equal probabilities of moving forward or backward according to the principle of detailed balance. Therefore, unidirectional motion is possible if the machine is driven out of equilibrium by some chemical, electrochemical, electronic, or photonic energy source [2–7]. In performing the conversion of energy into motion, the molecular machine is the stage of dissipative processes taking place at the nanoscale in the presence of thermal fluctuations. Accordingly, molecular machines are ruled not only by the laws of thermodynamics but also by the statistical laws of fluctuations ubiquitous at the nanoscale.

Remarkably, major results have been recently discovered which combine the thermodynamic and statistical laws into general relationships valid in nonequilibrium nanosystems such as molecular machines. Our purpose is here to give a brief summary of these new results which are called fluctuation theorems and nonequilibrium work relations, and to illustrate their application to the F$_{1}$-ATPase molecular motor.

II. FLUCTUATION THEOREM

Several versions of the fluctuation theorem have been proved for different types of situations [8–17]. This theorem rules the fluctuating fluxes flowing through a system driven out of equilibrium by thermodynamic forces, also called De Donder affinities [18–20]. The affinities are defined as the free enthalpy changes of the reactions powering the machine from the outside: $A_\gamma = \Delta G_\gamma = G_\gamma - G_{\gamma,eq}$. They are not fluctuating because they are fixed by the concentrations of the chemical species in large amounts in the environment of the molecular machine. The affinities are associated with the catalytic cycles driving the machine [21]. These thermodynamic forces generate fluctuating fluxes $J_\gamma$, which can be the rates of chemical reactions, the velocity of a linear molecular motor, the rotation rate of a rotary molecular motor, or the electric current in conducting devices. In the case of a chemical reaction, the flux is given by $J_\gamma = (1/t)\Delta N_\gamma(t)$ in terms of the number $\Delta N_\gamma(t)$ of molecules transformed by the reaction over the time interval $t$.

The fluctuation theorem asserts that the probability distribution $P\{\{J_\gamma\}\}$ of the fluxes in some statistically stationary regime is larger than the probability $P\{\{-J_\gamma\}\}$ of finding the opposite values of the fluxes, by a factor growing exponentially with the time $t$ and the magnitudes of the affinities and the fluxes according to:

$$\frac{P\{\{J_\gamma\}\}}{P\{\{-J_\gamma\}\}} = \exp\left(\frac{t}{k_B T} \sum_\gamma A_\gamma J_\gamma\right) \quad \text{for } t \to \infty$$

where $k_B$ is Boltzmann’s constant and $T$ the temperature [12, 15]. At equilibrium where the affinities vanish, detailed balance between forward and reversed fluctuations is recovered. We notice that the rate of exponential growth is equal on average to the thermodynamic entropy production:

$$\left.\frac{dS}{dt}\right|_{st} = \frac{1}{T} \sum_\gamma A_\gamma \langle J_\gamma \rangle \geq 0$$

(2)
where \( \langle \cdot \rangle \) denotes the statistical average in the stationary regime [18–20]. This shows that the fluctuation theorem deeply relates the statistics of fluctuations to the second law of thermodynamics. The surprising result is that the fluctuation theorem is valid away from equilibrium and allows us to derive new relationships extending Onsager’s reciprocity relations to the nonlinear response properties [12, 16]. This theorem also applies to ion channels and the counting statistics of electrons through quantum dots and single molecules [22, 23].

### III. NONEQUILIBRIUM WORK RELATIONS

Further results have been obtained for systems driven by some time-dependent control parameter \( \lambda(t) \) varying between \( \lambda_1 \) and \( \lambda_2 \). This parameter can be an external force as in AFM experiments unfolding mechanically a single molecule of RNA [24]. Because of the fluctuations, the work \( W \) performed on the molecule is statistically distributed. The probability distribution \( P_F(W) \) of the work performed during forward driving \( \lambda_1 \rightarrow \lambda_2 \) can be compared with the distribution \( P_R(W) \) corresponding to the reversed driving \( \lambda_2 \rightarrow \lambda_1 \), starting in both cases from their respective equilibrium canonical distribution at the temperature \( T \). Their ratio is ruled by Crooks’ fluctuation theorem [25]

\[
P_F(W) = \exp \left( \frac{W - \Delta F}{k_B T} \right) \quad (3)
\]

where \( \Delta F = F_2 - F_1 \) is the free energy difference between the thermodynamic equilibria at \( \lambda_2 \) and \( \lambda_1 \). A consequence is Jarzynski’s nonequilibrium work theorem [26]

\[
\langle \exp \left( - \frac{W}{k_B T} \right) \rangle = \exp \left( - \frac{\Delta F}{k_B T} \right) \quad (4)
\]

allowing the measurement of free energy landscapes with single-molecule force spectroscopy [27]. Extensions to quantum systems have also been obtained [28]. Moreover, Jarzynski’s theorem implies Clausius’ thermodynamic inequality:

\[
\langle W \rangle \geq \Delta F \quad (5)
\]

Furthermore, the mean work performed on the molecular machine is given by [29]

\[
\langle W \rangle = \Delta F + k_B T \int dq dp \rho_F(q, p, t) \ln \frac{\rho_F(q, p, t)}{\rho_R(q, -p, t)} \quad (6)
\]

in terms of the phase-space probability distributions of the positions \( q \) and momenta \( p \) of the particles at some intermediate time \( t \) during the aforementioned protocol. The equality (6) of statistical mechanics nicely completes Clausius’ thermodynamic inequality (5).

### IV. APPLICATION TO THE F\(_1\)-ATP\textsubscript{ase} MOLECULAR MOTOR

ATP is synthesized by rotational catalysis in the F\(_1\) part of mitochondrial F\(_{1}\)F\(_{0}\)-ATPase [30–32]. The F\(_1\) protein complex is a barrel composed of three large \( \alpha\)- and \( \beta\)-subunits circularly arranged around a smaller \( \gamma\)-subunit playing the role of the shaft. Professor Kinosita and coworkers have succeeded to observe the rotation of the shaft with a resolution into substeps of about 90\(^\circ\) upon ATP binding followed by the release of ADP and P\(_i\) during an extra rotation of about 30\(^\circ\) [33, 34]. Therefore, a full revolution of 360\(^\circ\) corresponds to the hydrolysis of three ATP molecules. The chirality (handedness) of the molecular complex is essential for its unidirectional rotation under nonequilibrium conditions. Compared to molecular machines such as rotaxanes and catenanes [2], the F\(_1\) molecular motor is much heavier and its large molecular architecture allows the separation of the catalytic sites in the \( \beta\)-subunits from the rotating \( \gamma\)-subunit. Nevertheless, the F\(_1\) motor remains affected by the thermal fluctuations. According to the fluctuation theorem [35], the probability of backward substeps is given by \( P(-s) = P(s) \exp \left[ -s A/(6k_B T) \right] \) in terms of the affinity

\[
A = -3 \Delta G^0 + 3 k_B T \ln \frac{[ATP]}{[ADP][P_i]} \quad (7)
\]

with the standard free enthalpy of hydrolysis \( \Delta G^0 \approx -50 \) pN nm at pH 7 and 23\(^\circ\)C [34]. The cycle of the motor corresponds to the full revolution with \( s = 6 \) substeps. Under physiological conditions, the motor runs in a highly nonlinear regime with a Michaelis-Menten kinetics and an affinity \( A \gtrsim 40 k_B T \) [35, 36]. In this regime, the fluctuation theorem shows that the backward substeps are very rare, explaining that unidirectional motion can overwhelm erratic Brownian motion as the motor is driven away from equilibrium.
V. PERSPECTIVES

The new results provide the foundations for a statistical thermodynamics of nonequilibrium nanosystems, allowing us to describe the unidirectional motion of molecular machines. These machines can be driven by time-dependent control parameters or autonomously along a catalytic cycle. In both cases, the unidirectional motion is possible thanks to the nonequilibrium drive by some energy source and the efficiency of the energy conversion can be characterized. During the reactions, the molecular machine undergoes a cycle of intramolecular transformations, in which its 3D structure changes with time [37]. Most remarkably, the new results of statistical thermodynamics explain that temporal ordering such as unidirectional motion can be the feature of systems driven out of equilibrium, showing that the second law of thermodynamics can generate dynamical order and information [38]. We can foresee future applications of the new results to molecular machines; to further single-molecule pulling experiments on RNA, DNA, proteins, and other polymers to determine their free-energy landscapes; or to the counting statistics of single-molecule reactive events possibly with the techniques of single-molecule fluorescence spectroscopy [39].

Note added after the conference. Since December 2007, several reviews have been published on the fluctuation theorems and their implications [40–43]. In the light of the fluctuation theorems, significant advances have been carried out in our understanding of the nonlinear response properties of single and coupled transport processes [44–50]. The chemomechanical coupling of molecular motors such as kinesin has been studied on the basis of the fluctuation theorem for the two currents which are the velocity and the ATP hydrolysis rate [51–53] and by using network representations of the motor dynamics [54, 55]. In a related context, fluctuating copolymerization processes have been shown to generate information thanks to the directionality provided by nonequilibrium conditions [56, 57]. Moreover, anholonomy in cyclically driven stochastic processes has been investigated [58–60], which is relevant to experiments with catenane or rotaxane molecules in chemical environments undergoing slow cyclic changes induced by external parameters [6, 61, 62].

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