Entropy production for mechanically or chemically driven biomolecules

Tim Schmiedl, Thomas Speck, and Udo Seifert

II. Institut für Theoretische Physik, Universität Stuttgart, 70550 Stuttgart, Germany

Abstract

Entropy production along a single stochastic trajectory of a biomolecule is discussed for two different sources of non-equilibrium. For a molecule manipulated mechanically by an AFM or an optical tweezer, entropy production (or annihilation) occurs in the molecular conformation proper or in the surrounding medium. Within a Langevin dynamics, a unique identification of these two contributions is possible. The total entropy change obeys an integral fluctuation theorem and a class of further exact relations, which we prove for arbitrarily coupled slow degrees of freedom including hydrodynamic interactions. These theoretical results can therefore also be applied to driven colloidal systems. For transitions between different internal conformations of a biomolecule involving unbalanced chemical reactions, we provide a thermodynamically consistent formulation and identify again the two sources of entropy production, which obey similar exact relations. We clarify the particular role degenerate states have in such a description.

PACS numbers:
05.40.-a Fluctuation phenomena, random processes, noise, and Brownian motion,
05.70.-a Thermodynamics,
82.39.-k Chemical kinetics in biological systems,
87.15.-v Biomolecules: structure and physical properties
I. INTRODUCTION

Biological systems are generically out of equilibrium. Still, for most processes in cell biology taking place on the level of a single (or few) molecules, the intracellular aqueous solution provides an environment with constant temperature. The genuine source of non-equilibrium are not temperature gradients but rather mechanical or chemical stimuli provided by external forces or imbalanced chemical reactions. Such a characterization motivates the quest for a thermodynamical understanding of mechanically or chemically driven non-equilibrium processes taking into account their necessarily stochastic character on the level of few molecules [1]. Crucial for such a program are consistent formulations of the first and the second law under these conditions.

For mechanically driven processes, the controlled unfolding of proteins, RNA, and DNA typically described by Langevin equations can serve as a paradigm (for a review, see Ref. [2]). For the overdamped motion of a single colloidal degree of freedom, Sekimoto has shown how to relate work, internal energy and exchanged heat with the terms occurring in the Langevin equation, thus providing a formulation of the first law on the level of a single trajectory [3]. The extension of this interpretation to a biomolecule with several overdamped spatial degrees of freedom subject to both a potential of mean force and some additional mechanical force applied via an AFM or optical tweezers is, in principle, straightforward and will be given below. As a refinement of the second law, the Jarzynski relation expresses the free energy difference of an initial (folded) and a final (unfolded) state by an exponential average of the non-equilibrium work spent in such a transition [4–7]. This relation has found widespread attention both in experimental and theoretical studies of unzipping and unfolding transitions [8–13]. It has also inspired theoretical studies on the probability distribution of the work spent in such processes [14–16]. Even though the Jarzynski relation does not explicitly require a definition of entropy on the level of a single trajectory, one obtains a second-law like inequality for the average work as a mathematical consequence. The concept of an entropy of a single trajectory is fruitful since it allows to derive equalities different from but related to the Jarzynski relation for the total entropy change directly [17].

For chemically driven processes, an equally comprehensive understanding and formulation is not yet available. Based on classical work on network thermodynamics [18–21], ensemble properties like mean heat dissipation or entropy production rate have been identified and investigated (see [22–25] and references therein) with only a few attempts to provide a thermodynamic interpretation of the single reaction events [26, 27]. Taking the Langevin equation for mechanically driven processes as a guideline, however, it should be possible to formulate for single biochemical reaction events a first-law like energy conservation statement. Likewise, for a proper formulation and refinement of the second law, one should develop a notion of entropy along such a single stochastic history of reaction events. Only after averaging one will then recover previous ensemble formulations. The motivation for such a trajectory-based approach also derives from the exciting experimental possibilities to study conformational changes of single enzymes using fluorescence spectroscopy as reviewed in Refs. [28, 29]. Finally, molecular motors comprise a class of systems where biochemical reactions lead to discrete mechanical steps for which such a thermodynamic modeling should become appropriate as well [30–37].

This paper presents a coherent theoretical framework for describing both mechanically or chemically driven transitions between different configurational internal states of a biomolecule in a way that is thermodynamically consistent on the level of a single tra-
jectory. In particular, concerning entropy production, we exploit the general framework introduced in Ref. [17] for such isothermal non-equilibrium processes. In Section II, we consider the mechanically driven dynamics of a biomolecule involving several (slow) degrees of freedom. We provide a first law-like interpretation of the Langevin equation for its coupled overdamped degrees of freedom and derive exact relations on entropy production along such a driven trajectory, thereby extending our previous work both to many degrees of freedom and to (long-range) hydrodynamic interactions among them. Such interactions will become particularly relevant for colloidal systems (to which the same formalism is applicable) if extant studies of their non-equilibrium thermodynamics [38–44] are pushed beyond the one particle level. In Section III, we first consider transitions between different internal states of a protein or enzyme caused by biochemical reactions involving unbalanced chemical species which are the source of non-equilibrium in this case. We then apply the general notion of entropy production introduced in Ref. [17] to such transitions and derive exact relations for the total entropy production. Finally, we discuss the modifications arising from a possible degeneracy of the states occurring in such a description. In Section IV, we discuss a few perspectives of our approach. The Appendix contains the path-integral based proof of a general integral fluctuation theorem for (hydro)dynamically coupled degrees of freedom in a time-dependent potential.

II. MECHANICALLY DRIVEN CASE

We describe the biomolecule by a set $x \equiv (x_1, \ldots, x_d)$ of internal coordinates, which should comprise the relevant $d$ slow degrees of freedom. In equilibrium, this molecule feels a potential (of mean force) $V_0(x)$. Optical tweezers or a cantilever attached via a linker give rise to an additional potential $V_{\text{ex}}(x, \lambda)$. The external control parameter $\lambda(\tau)$ describes the time-dependent motion of the tweezer focus or the base of the cantilever, see Fig. 1. As equation of motion, we choose a Langevin description

$$\dot{x}_i = -\mu_{ij} \frac{\partial V}{\partial x_j} + \zeta_i,$$

where summation over repeated indices is understood throughout the paper. Here $V(x, \lambda) \equiv V_0(x) + V_{\text{ex}}(x, \lambda)$ is the sum of both potentials. We allow for a non-diagonal mobility $\mu_{ij}(x)$ which can include hydrodynamic interactions, e.g., through an Oseen tensor [45]. The stochastic increments $\zeta_i$ are modeled as Gaussian white noise with

$$\langle \zeta_i(\tau) \zeta_j(\tau') \rangle \equiv 2\mu_{ij}(x)\delta(\tau - \tau').$$

Throughout the article, we measure energies in units of $k_B T$, which is set to 1. Likewise, we use a dimensionless entropy, i.e., we set the Boltzmann constant $k_B$ to 1 as well. Under equilibrium conditions for constant $\lambda$, the type of correlations (2) guarantees that the Boltzmann distribution $p(x, \lambda) \sim \exp[-V(x, \lambda)]$ is stationary. It is an essential assumption for the theory we will be discussing that these correlations persist despite the fact that for a time-dependent protocol $\lambda(\tau)$ we are no longer in equilibrium.

The Langevin dynamics can be cast in the form of the first law, i.e., energy conservation along a stochastic trajectory [3]. Manipulating the system by changing the external control parameter $\lambda$ gives rise to an increment in applied work

$$dw \equiv \frac{\partial V}{\partial \lambda} d\lambda.$$

3
This work will either change the internal energy
\[ dV = \frac{\partial V}{\partial x_i} dx_i + \frac{\partial V}{\partial \lambda} d\lambda \] (4)
or is dissipated as heat
\[ dq = dw - dV = -\frac{\partial V}{\partial x_i} dx_i \] (5)
into the thermal environment. Since the heat bath has constant temperature, we can identify this exchanged heat with a change in entropy of the medium as
\[ \dot{s}_m(\tau) = \frac{dq}{d\tau} = -\frac{\partial V}{\partial x_i} \dot{x}_i. \] (6)

This quite natural definition of the entropy change of the medium along each trajectory raises the question whether there is a corresponding entropy change of the biomolecule itself.

Following the route outlined in Ref. [17], we now show that such an entropy of the “system” can consistently be defined along each stochastic trajectory \( x(\tau) \) as
\[ s(\tau) \equiv -\ln p(x(\tau), \tau), \] (7)
where \( p(x, \tau) \) is the solution of the Fokker-Planck equation for the probability distribution
\[ \partial_t p(x, \tau) = -\partial_j j_i (x, \tau) = \frac{\partial}{\partial x_i} \mu_{ij} \left[ \frac{\partial V}{\partial x_j} + \frac{\partial}{\partial x_j} \right] p(x, \tau). \] (8)

Upon averaging with \( p(x, \tau) \), this stochastic entropy becomes the non-equilibrium Gibbs or Shannon entropy
\[ S(\tau) \equiv \langle s(\tau) \rangle = -\int d^d x \ p(x, \tau) \ln p(x, \tau). \] (9)
The advantage of defining such a system entropy is that one can prove quite general theorems involving the total entropy change

\[ \Delta s_{\text{tot}} \equiv s(t) - s(0) + \int_0^t d\tau \dot{s}_m(\tau) \]  

along a stochastic trajectory \( x(\tau) \) of length \( t \). As shown in Appendix A, this total entropy change obeys the integral fluctuation theorem

\[ \langle \exp[-\Delta s_{\text{tot}}] \rangle = 1, \]  

which implies immediately the second law in the form

\[ \langle \Delta s_{\text{tot}} \rangle \geq 0. \]  

The brackets \( \langle \cdots \rangle \) denote the average over infinitely many realizations of the process. Moreover, for any function of the final coordinates \( f(x_t) \) one even has the relation

\[ \langle f(x_t) \exp[-\Delta s_{\text{tot}}] \rangle = \langle f(x_t) \rangle. \]  

The relations (11) and (13) are quite universal since they hold for the non-equilibrium average \( \langle \cdots \rangle \) with any initial distribution \( p(x,0) \), for any trajectory length \( t \), and for any driving protocol \( \lambda(\tau) \).

These relations should be distinguished from both the Jarzynski relation [4, 5]

\[ \langle \exp[-W_d] \rangle = 1 \]  

and the relation [7]

\[ \langle f(x_t) \exp[-W_d] \rangle = \langle f(x_t) \rangle_{\text{eq},\lambda(t)}, \]  

where \( W_d \equiv W - \Delta F = W - [F(\lambda(t)) - F(\lambda(0))] \) is the dissipated work involved in the non-equilibrium transition between the initial equilibrium state at \( \lambda(0) \) with free energy \( F(\lambda(0)) \) and the final state at \( \lambda(t) \) with free energy \( F(\lambda(t)) \). In particular, in relation (15) the average on the right hand side corresponds to an equilibrium average at the final value of the control parameter, whereas in (13) it is the average involving the actual probability distribution \( p(x,t) \). It is crucial to note that for Eqs. (14) and (15) the initial distribution has to be the thermal equilibrium distribution for \( \lambda(0) \) whereas in Eqs. (11) and (13) it is arbitrary.

Even though the motivation of this presentation is on biomolecules, it should be clear that the mechanically driven case discussed here applies exactly to colloidal particles coupled through direct or hydrodynamically induced interactions and driven by time-dependent laser traps. For such systems, these theorems show that fluctuation theorems (as well as the Jarzynski relation) persist in the presence of hydrodynamic interactions.

### III. CHEMICALLY DRIVEN CASE

#### A. Enzyme or protein with internal states

As a model for a biomolecule driven by chemical forces, we consider a protein with \( M \) internal states \( \{1, 2, \ldots, M\} \). Each state \( n \) has internal energy \( E_n \). Transitions between
these states involve some other molecules $A_\alpha$, where $\alpha = 1, \ldots, N_A$ labels the different chemical species. We assume that the chemical potentials, i.e., the concentrations $c_\alpha$ of these molecules are controlled or clamped externally. A transition from state $n$ to state $m$ implies the reaction

$$\sum_\alpha r_{nm}^\alpha A_\alpha + n \xrightleftharpoons{w_{nm}} m + \sum_\alpha s_{nm}^\alpha A_\alpha. \tag{16}$$

Here, $r_{nm}^\alpha, s_{nm}^\alpha$ are the numbers of species $A_\alpha$ involved in this transition, see Fig. 2. We assume a dilute solution of $A_\alpha$ molecules in a solvent (modeled as a heat bath at constant temperature). Reaction time constants should thus be much larger than diffusion time constants. Hence, mass action law kinetics with respect to the $A_\alpha$ molecules is a good approximation and the ratio between forward rate $w_{nm}$ and backward rate $w_{mn}$ is given by

$$\frac{w_{nm}}{w_{mn}} = \frac{w_{nm}^0}{w_{mn}^0} \prod_\alpha (c_\alpha)^{r_{nm}^\alpha - s_{nm}^\alpha}. \tag{17}$$

Here, we separate the concentration dependence from some “intrinsic” or bare rates $w_{nm}^0, w_{mn}^0$. Their ratio can be determined by considering a hypothetical equilibrium condition for this reaction. In fact, if the reaction took place in equilibrium with concentrations $c_{\alpha}^{eq}$, we would have the detailed balance relation

$$\frac{w_{nm}^{eq}}{w_{mn}^{eq}} = \frac{w_{nm}^0}{w_{mn}^0} \prod_\alpha (c_{\alpha}^{eq})^{r_{nm}^\alpha - s_{nm}^\alpha} = \frac{p_{m}^{eq}}{p_{n}^{eq}} = \exp \left(-\Delta G \right), \tag{18}$$

where

$$\Delta G \equiv -[E_n - E_m + \sum_\alpha (r_{nm}^\alpha - s_{nm}^\alpha)\mu_\alpha^{eq}] \tag{19}$$

is the equilibrium free energy difference for this reaction and $p_{m,n}^{eq}$ are the equilibrium probabilities of states $m$ and $n$, respectively. The chemical potential for species $\alpha$ quite generally reads

$$\mu_\alpha \equiv E_\alpha + \ln c_\alpha \tag{20}$$

which for equilibrium becomes $\mu_\alpha^{eq} = E_\alpha + \ln c_{\alpha}^{eq}$. Combining this with Eqs. (18) and (19) shows that the ratio of the intrinsic rates

$$\frac{w_{nm}^0}{w_{mn}^0} = \exp[E_n - E_m + \sum_\alpha (r_{nm}^\alpha - s_{nm}^\alpha)\mu_\alpha] \tag{21}$$

involves only the energy-terms and is independent of concentrations. Eq. (17) for the ratio under non-equilibrium conditions then becomes

$$\ln \frac{w_{nm}}{w_{mn}} = E_n - E_m + \sum_\alpha (r_{nm}^\alpha - s_{nm}^\alpha)\mu_\alpha \equiv -\Delta E + w_{\text{chem}}^{nm}. \tag{22}$$

The right hand side corresponds to the difference between applied chemical work

$$w_{\text{chem}}^{nm} = \sum_\alpha (r_{nm}^\alpha - s_{nm}^\alpha)\mu_\alpha \tag{23}$$

(since every transformed $A_\alpha$ molecule gives rise to a chemical work $\mu_\alpha$) and the difference in internal energy $\Delta E$. For the first law to hold for this transition, we then have to identify
FIG. 2: Protein or enzyme with internal states. A forward transition (left) from \( n \) to \( m \) involves the chemical reaction \( A_1 + n \rightarrow m + A_2 + A_3 \) and similarly for the backward reaction (right). The rates \( w^0_{nm} \) and \( w^0_{mn} \) are the (not concentration-dependent) bare rates.

the left hand side of Eq. (22) with the heat delivered to the medium, i.e. with the change in entropy of the medium

\[
\ln \frac{w_{nm}}{w_{mn}} = \Delta s_{nm}.
\]  

We now show that this identification between the ratio of the forward rate and the backward rate with the heat exchanged in this step and hence the change in entropy of the medium (arising here from an interpretation of a single reaction step in terms of the first law) fits into the general scheme of entropy production in stochastic dynamics introduced in Ref. [17].

B. Entropy production in stochastic network dynamics

We briefly recall the essential relations of Ref. [17] where entropy production was defined quite generally for a Markovian dynamics on a discrete set of states \( \{n\} \). Let a transition between discrete states \( n \) and \( m \) occur with a rate \( w_{nm}(\lambda) \), which depends on an externally controlled time-dependent parameter \( \lambda(\tau) \). The master equation for the time-dependent probability \( p_n(\tau) \) then reads

\[
\partial_\tau p_n(\tau) = \sum_{m \neq n} [w_{mn}(\lambda)p_m(\tau) - w_{nm}(\lambda)p_n(\tau)].
\]  

For any fixed \( \lambda \), there is a steady state \( p^*_n(\lambda) \) [19].

A stochastic trajectory \( n(\tau) \) starts at \( n_0 \) and jumps at times \( \tau_j \) from \( n_j^- \) to \( n_j^+ \) ending up at \( n_t \). As entropy along this trajectory, we have defined

\[
s(\tau) \equiv -\ln p_n(\tau),
\]  

where \( p_n(\tau) \) is the solution \( p_n(\tau) \) of the master equation (25) for a given initial distribution \( p_n(0) \) taken along the specific trajectory \( n(\tau) \). The rate of entropy flow into the medium is defined as

\[
\dot{s}_m(\tau) \equiv \sum_j \delta(\tau - \tau_j) \ln \frac{w_{n_j^- n_j^+}}{w_{n_j^- n_j^-}} \equiv \sum_j \delta(\tau - \tau_j) \Delta s_{n_j^- n_j^+},
\]
which leads to a change in the medium entropy along a trajectory of length $t$ as

$$
\Delta s_m = \int_0^t d\tau \dot{s}_m(\tau).
$$

The total entropy change

$$
\Delta s_{\text{tot}} \equiv s(t) - s(0) + \Delta s_m = \sum_j \ln \frac{p_{n^-}}{p_{n^+}} + \sum_j \ln \frac{w_{n^-}}{w_{n^+}}
$$

then obeys an integral fluctuation theorem

$$
\langle \exp[-\Delta s_{\text{tot}}] \rangle = 1,
$$

which implies the second law like statement

$$
\langle \Delta s_{\text{tot}} \rangle \geq 0.
$$

Likewise, one has in complete analogy to the mechanically driven case discussed above the extension

$$
\langle f(n_t) \exp[-\Delta s_{\text{tot}}] \rangle = \langle f(n_t) \rangle,
$$

where $f(n_t)$ is any function of the final state.

These results hold for the non-equilibrium average with arbitrary initial state, arbitrary time-dependent rates $w_{nm}(\lambda)$ caused, e.g., by time-dependent concentrations $c_n(\lambda)$, and any length $t$ of trajectories.

Even though the entropy definition for the system (26) and the medium (27) have been given in Ref. [17] purely formally (or at most in analogy with the mechanically driven case), this definition of the change in entropy of the medium (28) corresponds exactly to the one found in (24) for our biomolecular example derived on the basis of the kinetics together with the first law formulation along a trajectory. Crucial for this agreement, however, is the persistence of the relation (21) for the intrinsic rates in a non-equilibrium situation. In fact, this persistence corresponds to maintaining the correlations (2) in non-equilibrium in the mechanically driven case.

### C. Several molecules or equivalent internal states: Role of “degeneracy”

The definitions (26) and (27) for system entropy and entropy change of the medium are correct and consistent with the simple assumptions for the kinetics if $n$ and $m$ label single states. An important modification arises if several states are lumped into one label $n$.

As an example, consider the case of $N$ identical but spatially separable and hence in principle distinguishable molecules of the type discussed above each involved in reactions (16). If we can resolve only the numbers $n = (n_1, \ldots, n_M)$ of molecules which are in a particular state but cannot distinguish which of the $n_n$ equivalent molecules undergoes the transition from $n$ to $m$, the state space can now be labeled by $n$ with the constraint $\sum_{i=1}^M n_i = N$. Likewise, we could assume we have $N$ equivalent reaction sites lined up consecutively along a multi-domain protein where each site could be in any of the $M$ states, see Fig. 3.

We now denote the rate for a transition from $n$ to $n'$ with

$$
\delta_{im} = n_i - \delta_{im} + \delta_{im}
$$

8
FIG. 3: Sketch of a multi-domain protein with \( N \) equivalent consecutive “reaction sites”, each involving \( M \) (here 3) internal states. The number of sites which are in the internal state \( m \) is \( n_m \) (here 3).

by \( W_{nm}(n) \) and the corresponding backward rate as \( W_{mn}(n') \). Mass action law kinetics implies

\[
\frac{W_{nm}(n)}{W_{mn}(n')} = \frac{w_{nm} n_n}{w_{mn} (n_m + 1)}
\]

since the forward rate is enhanced by the factor \( n_n \) which counts the number of molecules in the state \( n \). Likewise, for the corresponding backward transition, any of the (then) \( n_m + 1 \) molecules in state \( m \) can jump. If one forward reaction takes place, the entropy change of the medium \( \Delta s^{nm'}_m \) is still given by

\[
\Delta s^{nm'}_m = E_n - E_m + (r^{nm}_\alpha - s^{nm}_\alpha) \mu_\alpha = \ln \frac{w_{nm}}{w_{mn}},
\]

since the first law for a single reaction event remains the same as above. On the other hand, by naive application of the general expression (24) as

\[
\Delta s^{nm'}_m = \ln \frac{W_{nm}(n)}{W_{mn}(n')} = \Delta s^{nm}_m + \ln \frac{n_n}{n_m + 1}
\]

one would obtain an additional term \( \ln [n_n / (n_m + 1)] \).

The solution of this apparent inconsistency requires an analysis of the entropy definition (26) in the case of degeneracy. In our example, the state \( n \) carries a degeneracy

\[
g_n = \frac{N!}{\prod_i n_i!}
\]

We now define the stochastic entropy of the state \( n \) not by (26) but rather by

\[
s(\tau) \equiv -\ln p_n(\tau) + s_n^0(\tau)
\]

with the “intrinsic” entropy

\[
s_n^0 \equiv \ln g_n
\]

determined by the degeneracy. For a single transition \( n \) to \( n' \) at time \( \tau \), the system entropy then changes according to

\[
\Delta s^{nm'} = \ln \frac{p_n(\tau)}{p_{n'}(\tau)} + \ln \frac{g_n'}{g_n} = \ln \frac{p_n(\tau)}{p_{n'}(\tau)} + \ln \frac{n_n}{n_m + 1}.
\]
If we use this modified definition of system entropy change (40) and the thermodynamically correct change in medium entropy (35), the total entropy production in a single step

\[ \Delta s_{nn}'_{\text{tot}} = \Delta s_{nn}' + \Delta s_{m}' = \ln \frac{p_n(\tau)}{p_{n'}(\tau)} + \ln \frac{W_{nm}(n)}{W_{mn}(n')} \]  

(41)

has the form of the right hand side of Eq. (29). Hence, the fluctuation theorems (30) and (32) even hold in the case of a degenerate state space.

Generalizing and summarizing this procedure, we modify the expression developed in Ref. [17] for the change of the medium entropy as

\[ \dot{s}_m(\tau) \equiv \sum_j \delta(\tau - \tau_j) \left[ \ln \frac{w_{n_j} n_{j}^+}{w_{n_j^+} n_j} - (s_{n_j^+}^0 - s_{n_j}^0) \right] , \]  

(42)

where the additional term in round brackets compensates for each jump the change in the degeneracy factor. In the example discussed above, we now get for the contribution of this transition to the change in medium entropy

\[ \Delta s_{m}' \equiv \ln \frac{W_{nm}(n)}{W_{mn}(n')} - \ln \frac{g_n}{g_{n'}} = \ln \frac{w_{nm}}{w_{mn}}, \]  

(43)

which is indeed the thermodynamically correct expression. Hence, the modified definitions (38) and (42) for system and medium entropy change in the presence of degeneracy are not only consistent with a first law-like energy conservation but also obey the fluctuation theorems. While we have identified the intrinsic entropy with the degeneracy, it is tempting to speculate that even for other sources of intrinsic entropy the definitions (38) and (42) remain meaningful.

**D. Detailed fluctuation theorem in the steady state**

The reaction network discussed above allows also for a genuine non-equilibrium steady state. Necessary for such a state are at least three internal states in order to have at least one cycle in the network, i.e. two essentially different reaction paths leading to the same final state. A non-equilibrium steady state can be obtained if it is possible to adjust the concentrations \( \{c_\alpha\} \) such that a net flux in the species \( A_\alpha \) occurs. Hence, the stationary state violates the detailed balance condition \( p_n w_{nm} = p_m w_{mn} \). For such non-equilibrium steady states a detailed fluctuation theorem

\[ p(-\Delta s_{\text{tot}}) = \exp\left[-\Delta s_{\text{tot}}\right]p(\Delta s_{\text{tot}}) \]  

(44)

holds with the present entropy definition for any length of the trajectory [17] thus extending previous results valid in the long-time limit [46–50].

**IV. SUMMARIZING PERSPECTIVES**

The thermodynamically consistent description of non-equilibrium processes of small systems developed in this paper paradigmatically relies on two central concepts. First, we
need a first-law like energy balance along the stochastic trajectory. While its form is pretty obvious in the mechanical case, it is less straightforward in the chemical case where it involves identifying the dissipated heat as the ratio of the forward and backward rate (up to a possible degeneracy correction). Second, the non-equilibrium dynamics has to be formulated in such a way that if it is restricted to the equilibrium concentrations it obeys detailed balance with the appropriate equilibrium distribution. This condition does not determine the non-equilibrium dynamics uniquely. Still, the present choice for the rates both in the mechanical and in the chemical case seems to be the “minimal” extension of the equilibrium rates. In fact, one could call such a dynamics an *isothermal non-equilibrium dynamics* since the notion of temperature of the surrounding heat bath still makes sense and serves to relate exchanged heat (occurring in the first law) with an entropy change of the medium (entering the second law). For this type of dynamics, entropy along a stochastic trajectory can consistently be defined such that (i) it reduces upon averaging to the usual non-equilibrium ensemble formulation; and (ii) together with the identification of the entropy change of the medium the total entropy change obeys exact relations from which a second law for the average follows trivially.

Combining the chemically driven with the mechanically driven case discussed here separately is straightforward. Along this line, one could then apply our concepts to models previously introduced to describe such coupled systems like in Refs. [51, 52] or the motor models mentioned in the introduction. Likewise, the chemically driven case discussed here for one (or several identical) reaction sites can be extended to a thermodynamically consistent theory of any small-scale biochemical reaction network as will be discussed elsewhere [53].

The theoretical framework developed in this paper is quite general. Leaving the appeal of exact relations aside, its significance for any specific system will depend on working out the particular details. Of special interest seem to be the distribution for the entropy changes of system, medium and their sum. Presumably only little can be said for these distribution in general since even for simple driven non-biological two-level systems these distributions can exhibit a quite rich structure [54]. For a simple three-state model of the rotary motor in the steady state, the exact distribution of the entropy change is available through mapping to an asymmetric random walk [27]. Numerical analysis of more sophisticated models should finally provide us with a better understanding of how entropy changes on the stochastic level look like beyond the exact constraints developed in this paper. Finally, it will be exciting to see when and how these elements of a non-equilibrium thermodynamics will be integrated to a consistent and comprehensive theory of the physics of the cell.

**APPENDIX A: PROOF OF INTEGRAL FLUCTUATION THEOREMS**

In this appendix we show how to extend proofs [7, 17, 50] of integral fluctuation relations based on time-reversal to many degrees of freedom involving hydrodynamic interactions. The integral fluctuation theorem for the total entropy production (11), the Jarzynski relation, and the more general relation (13) then all derive from one master formula, which has been given before for the one-dimensional case in Ref. [17].

Since the thermal noise $\zeta_i(\tau)$ in Eq. (1) is modeled as Gaussian noise, the probability for a noise trajectory is $P[\zeta(\tau)] = \mathcal{N} \exp\{-A[\zeta(\tau)]\}$ with “action”

$$A[\zeta(\tau)] \equiv \frac{1}{2} \int_0^t d\tau \int_0^t d\tau' \zeta_i(\tau) K^{-1}_{ij}(\tau - \tau') \zeta_j(\tau'), \quad (A1)$$
correlation matrix $K_{ij}(\tau - \tau') \equiv \langle \zeta_i(\tau)\zeta_j(\tau') \rangle$, and normalization $N$. We make the transition from the noise history $\zeta(\tau)$ to the trajectory $x(\tau)$ given the initial state $x_0$ by inserting the Langevin equation (1)

$$\dot{x}_i = -\mu_{ij}(x) \frac{\partial V}{\partial x_j}(x, \lambda(\tau)) + \zeta_i \equiv v_i(x, \tau) + \zeta_i$$

(A2)

along with the noise correlation (2) into Eq. (A1), leading to

$$A[\zeta(\tau)] = \frac{1}{4} \int_0^t d\tau \ [\dot{x}_i(\tau) - v_i(x(\tau), \tau)] \mu_{ij}^{-1} [\dot{x}_j(\tau) - v_j(x(\tau), \tau)].$$

(A3)

The change of variables from $\zeta(\tau)$ to $x(\tau)$ also leads to a Jacobian $J[x(\tau)]$ in the trajectory weight. The Langevin equation discretized into $N$ steps takes the form

$$x_\alpha^i - x_{\alpha-1}^i \varepsilon = \frac{1}{2} \left[ v_i^\alpha(x^\alpha) + v_i^{\alpha-1}(x^{\alpha-1}) \right] + \zeta_i^\alpha,$$

(A4)

where the upper Greek indices represent discrete time and $\varepsilon$ is a small time step. This discretization corresponds to Stratonovich’s scheme. The Jacobian matrix resulting from the change of variables is

$$J_{ij}^{\alpha\beta} \equiv \frac{\partial \zeta_i^{\alpha}}{\partial x_j^\beta},$$

(A5)

from which we calculate the Jacobian as

$$J[x(\tau)] \equiv \lim_{\varepsilon \to 0} \det J_{ij}^{\alpha\beta}.$$  

(A6)

In order to see the structure of the Jacobian matrix, we define for a given time index $\alpha$

$$\pm M_{ij}^{\alpha} \equiv \pm \delta_{ij} - \varepsilon \frac{\partial v_i^{\alpha}}{2 \partial x_j}(x^\alpha) \approx \pm \left[ \exp \left\{ \pm \frac{\varepsilon}{2} \frac{\partial v_i^{\alpha}}{\partial x_j}(x^\alpha) \right\} \right]_{ij}.$$  

(A7)

The Jacobian matrix can then be written as matrix of matrices

$$J = \frac{1}{\varepsilon} \begin{pmatrix}
+M_1 & 0 & 0 & 0 \\
-M_1 & +M_2 & 0 & 0 \\
0 & -M_2 & +M_3 & 0 \\
0 & 0 & -M_3 & +M_4 \\
\end{pmatrix},$$

(A8)

from which the determinant immediately follows as

$$J[x(\tau)] = \lim_{\varepsilon \to 0} \varepsilon^{-Nd} \prod_{\alpha=1}^N \det M^{\alpha}.$$  

(A9)

Using the identity $\det \exp = \exp \text{tr}$ and after taking the limit $\varepsilon \to 0$, $N \to \infty$ with $N\varepsilon = t$ we finally arrive at

$$J[x(\tau)] = \exp \left( -\frac{1}{2} \int_0^t d\tau \sum_{ij} \frac{\partial v_i}{\partial x_j}(x(\tau), \tau) \right).$$  

(A10)
The action (A3) along a stochastic trajectory can be split into two contributions

\[ A_s[x(\tau)|x_0] = \frac{1}{4} \int_0^t d\tau \left\{ \dot{x}_i \mu_{ij}^{-1} \dot{x}_j + \frac{\partial V}{\partial x_i} \mu_{ij} \frac{\partial V}{\partial x_j} \right\}, \quad (A11) \]

\[ A_a[x(\tau)|x_0] = \frac{1}{2} \int_0^t d\tau \frac{\partial V}{\partial x_i} \dot{x}_i = -\frac{\Delta s_m}{2}, \quad (A12) \]

\[ A = A_s + A_a, \] where for the last equality we have used Eq. (6). Under time reversal, i.e., under the transformation

\[ \tau \mapsto t - \tau \equiv \bar{\tau} : \lambda(\tau) \mapsto \bar{\lambda}(\bar{\tau}), \quad x_i(\tau) \mapsto \bar{x}_i(\bar{\tau}), \quad \dot{x}_i(\tau) \mapsto -\dot{\bar{x}}_i(\bar{\tau}) \quad (A13) \]

the symmetric part of the action stays invariant, \( \bar{A}_s = A_s \), whereas \( \bar{A}_a = -A_a \) changes sign. Since the Jacobian \( J \) only involves mobility \( \mu \) and potential energy \( V \) it is invariant under time reversal, \( \bar{J} = J \). For given initial state \( x_0 \) and final state \( \bar{x}_0 = \bar{x}_0 \), the total trajectory weight becomes

\[ P[x(\tau)|x_0] = \mathcal{N} J[x(\tau)|x_0] \exp \left\{ -A_s[x(\tau)|x_0] - A_a[x(\tau)|x_0] \right\}, \quad (A14) \]

\[ \bar{P}[\bar{x}(\bar{\tau})|\bar{x}_0] = \mathcal{N} J[x(\tau)|x_0] \exp \left\{ -A_s[x(\tau)|x_0] + A_a[x(\tau)|x_0] \right\}. \quad (A15) \]

In order to prove a general version of the integral fluctuation theorem we combine the physical picture of time reversal with a generalization of the actual final distribution \( p(x_t) \) to an arbitrary normalized initial distribution \( p_1(x_0) \) for time-reversed paths. Normalization then implies

\[ 1 = \sum_{\bar{x}(\bar{\tau})} \bar{P}[\bar{x}(\bar{\tau})|\bar{x}_0] p_1(\bar{x}_0), \quad (A16) \]

where the summation runs over all trajectories. Inserting the actual initial distribution \( p_0(x_0) \) we have the master formula

\[ 1 = \sum_{\bar{x}(\bar{\tau})} \frac{\bar{P}[\bar{x}(\bar{\tau})|\bar{x}_0] p_1(\bar{x}_0)}{P[x(\tau)|x_0] p_0(x_0)} P[x(\tau)|x_0] p_0(x_0) = \left\langle \frac{\bar{P}[\bar{x}(\bar{\tau})|\bar{x}_0] p_1(\bar{x}_0)}{P[x(\tau)|x_0] p_0(x_0)} \right\rangle \equiv \langle \exp[-R] \rangle \quad (A17) \]

with

\[ R = \ln \frac{P[x(\tau)|x_0] p_0(x_0)}{\bar{P}[\bar{x}(\bar{\tau})|\bar{x}_0] p_1(\bar{x}_0)} = -\ln \frac{p_1}{p_0} + \Delta s_m. \quad (A18) \]

Replacement of \( \sum_{\bar{x}} \) by \( \sum_x \) is admissible since it does not matter how we denote the summation variable when we sum over all trajectories.

For the proof of Eq. (11), we choose with \( p_1(x) = p(x,t) \) the actual probability distribution at the end of the trajectory. With \( p_0(x) \) the distribution of the initial state, the ratio

\[ R = \Delta s + \Delta s_m = \Delta s_{\text{tot}} \quad (A19) \]

becomes the total change of entropy. If we choose the normalized function

\[ p_1(x) = \frac{f(x)p(x,t)}{\langle f(x) \rangle} \quad (A20) \]
with an arbitrary function $f(x)$, where the average in the denominator implies the distribution $p(x,t)$, the ratio becomes

$$R = \Delta s_{\text{tot}} - \ln \frac{f(x)}{\langle f(x) \rangle},$$

leading to Eq. (13). Finally, if one chooses $p_1(x) = p_{\text{eq}}(x,\lambda(t))$, one obtains the Jarzynski relation (14) and analogously the general relation (15).

[1] C. Bustamante, J. Liphardt, and F. Ritort, Physics Today 58, 43 (2005).
[2] F. Ritort, Sém. Poincaré 2, 63 (2003).
[3] K. Sekimoto, Prog. Theor. Phys. Supp. 130, 17 (1998).
[4] C. Jarzynski, Phys. Rev. Lett. 78, 2690 (1997).
[5] C. Jarzynski, Phys. Rev. E 56, 5018 (1997).
[6] G. E. Crooks, Phys. Rev. E 60, 2721 (1999).
[7] G. E. Crooks, Phys. Rev. E 61, 2361 (2000).
[8] G. Hummer and A. Szabo, Proc. Natl. Acad. Sci. U.S.A. 98, 3658 (2001).
[9] J. Liphardt, S. Dumont, S. B. Smith, I. Tinoco Jr, and C. Bustamante, Science 296, 1832 (2002).
[10] O. Braun, A. Hanke, and U. Seifert, Phys. Rev. Lett. 93, 158105 (2004).
[11] S. Park and K. Schulten, J. Chem. Phys. 120, 5946 (2004).
[12] D. Collin, F. Ritort, C. Jarzynski, S. Smith, I. Tinoco, and C. Bustamante, Nature 437, 231 (2005).
[13] T. Speck and U. Seifert, Eur. Phys. J. B 43, 543 (2005).
[14] T. Speck and U. Seifert, Phys. Rev. E 70, 66112 (2004).
[15] A. Imparato and L. Peliti, Europhys. Lett. 69, 643 (2005).
[16] A. Imparato and L. Peliti, Europhys. Lett. 70, 740 (2005).
[17] U. Seifert, Phys. Rev. Lett. 95, 040602 (2005).
[18] G. Oster, A. Perelson, and A. Katchalsky, Nature 234, 393 (1971).
[19] J. Schnakenberg, Rev. Mod. Phys. 48, 571 (1976).
[20] T. L. Hill, Free Energy Transduction and Biochemical Cycle Kinetics (Dover, 1989), 2nd ed.
[21] G. Nicolis and I. Prigogine, Self-Organization in Nonequilibrium Systems : From Dissipative Structures to Order through Fluctuations (Wiley, 1977).
[22] P. Gaspard, J. Chem. Phys. 120, 8898 (2004).
[23] D. Andrieux and P. Gaspard, J. Chem. Phys. 121, 6167 (2004).
[24] H. Qian and D. A. Beard, Biophys. Chem. 114, 213 (2005).
[25] H. Qian, J. Phys.: Condens. Matter 17, S3783 (2005).
[26] T. Shibata, cond-mat/0012404 (2000).
[27] U. Seifert, Europhys. Lett. 70, 36 (2005).
[28] P. Schwille, Cell Biochem. Biophys. 34, 383 (2001).
[29] X. S. Xie, J. Chem. Phys. 117, 11024 (2002).
[30] H. Qian, Biophys. Chem. 67, 263 (1997).
[31] F. Jülicher, A. Adjari, and J. Prost, Rev. Mod. Phys. 69, 1269 (1997).
[32] M. E. Fisher and A. B. Kolomeisky, Proc. Natl. Acad. Sci. U.S.A. 96, 6597 (1999).
[33] R. Lipowsky, Phys. Rev. Lett. 85, 4401 (2000).
[34] H. Qian, Phys. Rev. E 64, 022101 (2001).
[35] P. Reimann, Phys. Rep. 361, 57 (2002).
[36] C. Maes and M. H. van Wieren, J. Stat. Phys 112, 329 (2003).
[37] J. E. Baker, J. Theor. Biol. 228, 467 (2004).
[38] T. Hatano and S. Sasa, Phys. Rev. Lett. 86, 3463 (2001).
[39] G. M. Wang, E. M. Sevick, E. Mittag, D. J. Searles, and D. J. Evans, Phys. Rev. Lett. 89, 50601 (2002).
[40] D. M. Carberry, J. C. Reid, G. M. Wang, E. M. Sevick, D. J. Searles, and D. J. Evans, Phys. Rev. Lett. 92, 140601 (2004).
[41] R. van Zon and E. G. D. Cohen, Phys. Rev. Lett. 91, 110601 (2003).
[42] R. van Zon and E. G. D. Cohen, Phys. Rev. E 67, 46102 (2003).
[43] E. H. Trepagnier, C. Jarzynski, F. Ritort, G. E. Crooks, C. J. Bustamante, and J. Liphardt, Proc. Natl. Acad. Sci. U.S.A. 101, 15038 (2004).
[44] V. Blickle, T. Speck, L. Helden, U. Seifert, and C. Bechinger, Phys. Rev. Lett., in press (2006).
[45] M. Doi and S. F. Edwards, The Theory of Polymer Dynamics (Clarendon Press, Oxford, 1986).
[46] D. J. Evans and D. J. Searles, Phys. Rev. E 50, 1645 (1994).
[47] G. Gallavotti and E. G. D. Cohen, Phys. Rev. Lett. 74, 2694 (1995).
[48] J. Kurchan, J. Phys. A: Math. Gen. 31, 3719 (1998).
[49] J. L. Lebowitz and H. Spohn, J. Stat. Phys 95, 333 (1999).
[50] C. Maes, Sém. Poincaré 2, 29 (2003).
[51] H. Qian, J. Phys. Chem. B 106, 2065 (2002).
[52] O. Braun and U. Seifert, Eur. Phys. J. E 18, 1 (2005).
[53] T. Schmiedl and U. Seifert, in prep.
[54] S. Schuler, T. Speck, C. Tietz, J. Wrachtrup, and U. Seifert, Phys. Rev. Lett. 94, 180602 (2005).

15