Stochastic thermodynamics of single enzymes and molecular motors

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Abstract. For a single enzyme or molecular motor operating in an aqueous solution of non-equilibrated solute concentrations, a thermodynamic description is developed on the level of an individual trajectory of transitions between states. The concept of internal energy, intrinsic entropy and free energy for states follows from a microscopic description using one assumption on time-scale separation. A first law energy balance then allows the unique identification of the heat dissipated in one transition. Consistency with the second law on the ensemble level enforces both stochastic entropy as third contribution to the entropy change involved in one transition and the local detailed balance condition for the ratio between forward and backward rates for any transition. These results follow without assuming weak coupling between the enzyme and the solutes, ideal solution behavior or mass action law kinetics. The present approach highlights both the crucial role of the intrinsic entropy of each state and the physically questionable role of chemostats for deriving the first law for molecular motors subject to an external force under realistic conditions.

PACS. 05.70.Ln Nonequilibrium and irreversible thermodynamics – 87.16.Uv Active transport processes

1 Introduction

Conformational changes of single enzymes have become observable through a variety of methods often summarized as single molecule techniques [1,2]. Typically, such an enzyme is embedded in an aqueous solution containing different solutes at specified concentrations. Such a preparation, despite having a well-defined temperature, often leads to a non-equilibrium system since the aqueous solution is not in equilibrium with respect to chemical reactions catalyzed by the enzyme. Moreover, for the case of motor proteins [3,4], time-dependent external forces arising if beads in optical traps are connected via polymeric spacers to the enzyme provide a source of non-equilibrium. Taking seriously both the single molecule set-up showing individual transitions and the thermodynamic description of the surrounding solution then prompts the question whether, and if yes how, the thermodynamic laws can be applied to such processes on the single molecule level. Is it possible to identify the amount of heat released into (or taken up from) the aqueous solution constituting an effective heat bath if a molecular motor advances one step? And how much entropy is produced in such a single step?

The conceptual and technical tools required for such an approach on the level of individual trajectories have been developed under the label of “stochastic energetics” [5], “thermodynamics of small systems” [6], or, if entropy production is included, “stochastic thermodynamics” [7], recently. The basic idea, indeed, is to formulate, on the level of an individual trajectory, both a first law, i.e. an energy conserving balance between an appropriately defined external work, internal energy and dissipated heat [8], and to identify entropy production [9].

The paradigm for such a trajectory based approach are colloidal particles in time-dependent potentials created by various forms of laser traps. Several joint studies between experiments and theory have illustrated how the thermodynamic quantities can be extracted from records of the fluctuating trajectories of such driven Brownian motion [10,11,12,13,14,15]. In a slight generalization, (bio)polymers with their internal shape degrees of freedom have been subject to a similar analysis some of which directed to deduce free energy landscapes from non-equilibrium experiments [16,17,18,19,20,21,22,23]. In both cases, the theoretical description typically is based on Langevin-type dynamics.

Molecular motors have also been modelled using Langevin dynamics in a potential that depends explicitly on the current chemical state of the motor [24,25,26,27]. Alternatively, as often used for enzymes, a description based on discrete, distinguishable states between which (sudden) transitions take place is often more appropriate [28,29,30,31,32,33,34,35,36,37,38,39,40,41]. In most of these works the focus has been on elucidating the cycles involved in the action of the motor and on deriving force-velocity curves and their dependence on ATP and ADP concentrations. A stochastic thermodynamics approach has been applied in Refs. [32,39] for deriving a fluctuation theorem and in...
The purpose of the present paper is to develop the stochastic thermodynamics of single enzymes and molecular motors afresh and coherently under minimal assumptions thus clearly dissecting conditions that are necessary from those which are (too) simplifying but not necessary. Since this work deals partially with topics addressed previously it is appropriate to point out the main differences and new results up front. First, some of the earlier work (and even recent ones [48]) does not distinguish carefully between internal energy and free energy of a state. Enzymes differ in this respect from simple colloidal particles which have no relevant internal degrees of freedom. Within stochastic thermodynamics the first recognition of this aspect seems to have been our discussion of the role of degenerate states [43] but a more systematic approach starting from a solid microscopic model is appropriate. A consequence of the correct treatment is that the heat released in one step can no longer be inferred from the rates directly as it can in the case of a colloidal particle. Moreover, formulated correctly, heat in the stochastic thermodynamics approach becomes unique and identical to the caloric one thereby remedying a deficiency pointed out by Sekimoto [34]. Second, the thermodynamics of molecular motors has previously been described using the concept of "chemiostats" [34,35,36,37,38]. We show that the heat thus identified would require rather unrealistic experimental conditions and should, in practice, be replaced by a new expression derived below. Third, in our earlier work [43,47], explicit expressions for the individual transition rates based on mass action law kinetics have been invoked early on thus effectively restricting the range of applicability unnecessarily. In fact, the first law can be formulated on the trajectory level without any assumptions on the transition rates. Finally, we now can show that the correct identification of entropy production occurring in one step follows quite naturally from requiring consistency with the second law on the ensemble level. In particular, this condition leads to both stochastic entropy [41] as a necessary third contribution to the entropy change associated with one transition and to an expression for the ratio of the forward and backward rate known as local detailed balance. The latter property is usually either postulated or claimed to follow from microreversibility which is a concept not so trivially applicable to chemical transformations under non-equilibrium conditions.

This paper is organized as follows. In Sect. 2, we set the stage, define thermodynamic notions for the states of an enzyme undergoing only conformational changes and not yet catalyzing reactions. In Sect. 3, we discuss the modifications required if we allow chemical reactions. In Sect. 4, we apply this formalism to molecular motors. Sect. 5 deals with entropy production for all these systems. A brief summary and conclusions follows in Sect. 6. In the appendix, we discuss the implications of our trajectory-based approach for the thermodynamics of time-dependent ensembles.

2 Configurational transitions of a single enzyme

2.1 Solution

The enzyme will be placed in an aqueous solution which consists of a set of \{N_i\} molecules of type \(i\) enclosed in a volume \(V\) at a temperature \(T\). The microscopic configurations, i.e., the micro-states, of this solution (without the enzyme yet) are labelled collectively by \(\xi^{\text{sol}}\). The configurational energy of the whole solution can be expressed by a potential \(V^{\text{sol}}(\xi^{\text{sol}})\) leading to the probability

\[
p(\xi^{\text{sol}}) = \exp[-\beta(V^{\text{sol}}(\xi^{\text{sol}}) + E^{\text{sol}})]\]

for each micro state \(\xi^{\text{sol}}\) with the free energy

\[
F^{\text{sol}} = -k_B T \ln \sum_{\xi^{\text{sol}}} \exp[-\beta V^{\text{sol}}(\xi^{\text{sol}})].
\]

Here \(\beta \equiv 1/k_B T\) is the inverse temperature and \(k_B\) Boltzmann’s constant. The (mean) internal energy of this solution is given by

\[
E^{\text{sol}} = \sum_{\xi^{\text{sol}}} p(\xi^{\text{sol}}) V^{\text{sol}}(\xi^{\text{sol}})
\]

and its entropy by

\[
S^{\text{sol}} = \sum_{\xi^{\text{sol}}} p(\xi^{\text{sol}}) \ln p(\xi^{\text{sol}}) = (E^{\text{sol}} - F^{\text{sol}})/T.
\]

All these quantities depend on \(T, V\) and \(\{N_i\}\). Moreover, we will assume that this solution is large enough to be treated in the thermodynamic limit which implies that the chemical potential for species \(i\),

\[
\mu_i = \frac{\partial N_i}{\partial V} F^{\text{sol}},
\]

becomes a function of \(T\) and the concentrations \(c_i \equiv \{N_i/V\} \) only.

2.2 Thermodynamic quantities of states

To this solution, we add a single enzyme, see Fig. 1. Following in spirit Hill’s classical work [50], we distinguish different (mesoscopic) states of the enzyme such that equilibration among microstates corresponding to the same state is fast whereas transitions between these states are assumed to be slower and observable. Under these conditions, we can assign to each state \(n\) a free energy \(F^{\text{enz}}_n\), an internal energy \(E^{\text{enz}}_n\), and an intrinsic entropy \(S^{\text{enz}}_n\) following in spirit Hill’s classical work [50].

We denote the microscopic configurational degrees of freedom of an enzyme with fixed position of its center of
mass collectively by \( \{ \xi_{\text{enz}} \} \). The full configurational energy of the system consisting of enzyme and solution becomes

\[
V_{\text{tot}}(\xi_{\text{enz}}, \xi_{\text{sol}}) \equiv V(\xi_{\text{sol}}) + V(\xi_{\text{enz}}, \xi_{\text{sol}}) \equiv V_{\text{tot}}(\xi),
\]

where \( V(\xi_{\text{enz}}, \xi_{\text{sol}}) \) contains both the interaction within the enzyme and the interaction between enzyme and solution. We now partition all microstates \( \{ \xi \} = \{ (\xi_{\text{enz}}, \xi_{\text{sol}}) \} \) of the combined system enzyme and solution into a set of state configurations \( \{ \mathcal{C}_n \} \) such that each microstate \( \xi \) of the combined system occurs in one and only one such set \( \mathcal{C}_n \). For any specific state \( n \), the probability \( p(\xi | n) \) of finding an allowed microstate of the combined system consisting of enzyme and solution then follows from the assumption of fast equilibration as

\[
p(\xi | n) = \exp[-\beta(V_{\text{tot}}(\xi) - F_n)]
\]

with \( \beta \equiv 1/k_B T \) and the constrained free energy in state \( n \)

\[
F_n \equiv -k_B T \ln \sum_{\xi \in \mathcal{C}_n} \exp[-\beta V_{\text{tot}}(\xi)]
\]

ensuring proper normalization \( \sum_{\xi \in \mathcal{C}_n} p(\xi | n) = 1 \). The (mean) internal energy in state \( n \) is

\[
E_n \equiv \sum_{\xi \in \mathcal{C}_n} p(\xi | n) V_{\text{tot}}(\xi)
\]

and the (intrinsic) entropy becomes as usually

\[
S_n = -k_B \sum_{\xi \in \mathcal{C}_n} p(\xi | n) \ln p(\xi | n) = (E_n - F_n)/T.
\]

The thus defined free energy, internal energy and (intrinsic) entropy of each state of the combined system will depend on \( T, V \) and \( \{ N_i \} \). For a finite range of the interaction potential \( V(\xi_{\text{enz}}, \xi_{\text{sol}}) \), we can indentify the free energy, internal energy and intrinsic entropy of the enzyme proper with

\[
F_n^{\text{enz}}(\{ c_i \}) \equiv F_n(\{ N_i \}) - F_{\text{sol}}(\{ N_i \}),
\]

and

\[
S_n^{\text{enz}}(\{ c_i \}) \equiv S_n(\{ N_i \}) - S_{\text{sol}}(\{ N_i \}),
\]

respectively. In the thermodynamic limit of the solution, these quantities become independent of system size and depend only on the concentrations \( \{ c_i \} \). Since we keep \( T \) and \( V \) fixed throughout the paper, we suppress the dependence on these quantities notionally and often that on \( \{ c_i \} \) as well.

Since both the full quantities as well as the bare solution quantities obey the usual relation between free energy, internal energy and entropy, it is obvious that

\[
F_n^{\text{enz}} = E_n^{\text{enz}} - T S_n^{\text{enz}}
\]

holds as well. So it looks like the full system, enzyme plus solution, could be split into two subsystems with additive thermodynamic quantities despite the fact that the enzyme is neither a macroscopic object nor that we have assumed that the interaction between enzyme and solution is in any sense small. The caveat, however, is that the quantities referring to the enzyme \( (F_n^{\text{enz}}, E_n^{\text{enz}} \text{ and } S_n^{\text{enz}}) \) will depend on the concentrations \( \{ c_i \} \) which refer primarily to properties of the solution.

2.3 First law

In the spirit of stochastic thermodynamics, we now formulate a first-law like energy balance for transitions between states. Obviously, there is no external work playing any role for such a closed system. If the enzyme jumps from state \( m \) to state \( n \), the change in internal energy

\[
\Delta E \equiv E_n - E_m = E_n^{\text{enz}} - E_m^{\text{enz}} = -q
\]

can be identified with an amount \( q \) of heat being released into (or, if negative, being taken up from) the surrounding heat bath.

3 Enzymatic reactions

3.1 Binding of substrate molecules

For an enzyme, configurational changes often involve the binding or release of smaller molecules from the surrounding solution like the nucleotides ATP, ADP or inorganic phosphate P_i. For states with such bound molecules, like the one shown in Fig. 1c, it will be convenient to identify the free energy of the state somewhat differently than done in Fig. 1a. In the case where state \( n \) has one A1 molecule tightly bound to it, we define

\[
F_n^{\text{enz}}(\{ c_i \}) = F_n^{\text{enz}}(\{ N_i \}) - F_{\text{sol}}(\{ N_i \}),
\]

where we use \( \{ \} \) and drop notationally the dependence on the irrelevant species \( \{ N_i \} \) with \( i \neq 1 \). This definition thus means that \( F_n^{\text{enz}} \) is obtained by subtracting from the total

Fig. 1. (a) Solution with two types of solutes. (b) Enzyme in solution in state \( m \). (c) Enzyme in state \( n \) with one solute molecule tightly bound, and, consequently one less molecule in solution. The dashed line in (b) and (c) reflects the purely formal splitting of the total system into the solution and the enzyme as indicated in (11) and (12) for the free energy of state \( m \) and state \( n \), respectively.
free energy $F_m$ of the combined system the free energy of a solution containing one less $A_1$ molecule (the bound one). The idea behind it is again a conceptual (but not physical) splitting of the whole system into the enzyme (plus the bound molecule) and the solution possible despite the fact that both may interact strongly.

The advantage of this scheme becomes obvious if we consider a binding event conventionally written as

$$m + A_1 ⇔ n$$

(17)

where upon binding of an $A_1$ molecule a state $m$ transforms into the state $n$ just discussed, compare Fig. 1 (b) and (c). The free energy difference involved in this process becomes

$$\Delta F = F_n - F_m$$

$$= (F_{m}^{enz} - \mu_1 + F_{sol}^{m}) - (F_m^{enz} + F_{sol}^{m})$$

$$= F_{m}^{enz} - F_m^{enz} - \mu_1.$$  (18)

If one changes the concentrations of $A_1$ molecules in the solution one would expect that the difference in free energies depends on this concentration. Such a concentrations dependence becomes obvious through the $\mu_1$ term. The free energy $F_{m}^{enz}$ defined according to (13) will typically only weakly depend on concentration since it mainly contains the interaction between the one bound $A_1$ and the enzyme. If we had used the definition (11) also for the free energy of the enzyme in state $n$, $\mu_1$ would not show up explicitly in (20). The dominant concentration dependence of the free energy change would then be hidden in the $F_{m}^{enz}$ term.

In a more general case, if a state $n$ has $\sum_i r_i A_i$ molecules bound to it, we define its free energy as

$$F_n^{enz}(\{c_i\}) \equiv F_n(\{N_i\}) - F_{sol}^{\{\{N_i - r_i\}\}}$$

$$= F_n(\{N_i\}) + \sum_i r_i \mu_i - F_{sol}^{\{\{N_i\}\}},$$

(21)

Likewise, one can identify the entropy and internal energy of the enzyme in such a state accordingly as

$$S_n^{enz}(\{c_i\}) \equiv S_n(\{N_i\}) - S_{sol}^{\{\{N_i - r_i\}\}}$$

$$= S_n(\{N_i\}) - \sum_i r_i \partial T \mu_i - S_{sol}^{\{\{N_i\}\}},$$

(23)

where we use

$$\partial N_i S_{sol} = -\partial T \mu_i,$$

(25)

and

$$E_n^{enz}(\{c_i\}) \equiv E_n(\{N_i\}) - E_{sol}^{\{\{N_i - r_i\}\}}$$

$$= E_n(\{N_i\}) +$$

$$+ \sum_i r_i(\mu_i - T \partial T \mu_i) - E_{sol}^{\{\{N_i\}\}},$$

(26)

So from now on for the precise definition of the free energy, internal energy and entropy of a state we need to distinguish between states that have solute molecules bound to them for which the definition (21-28) will be used from those which have not for which we will use (11-13).

While this distinction of identifying the quantities with the superscript “enz” may seem somewhat pedantic for pure binding reactions, it becomes mandatory when we consider enzymatic reactions.

### 3.2 Example: Hydrolysis of ATP

For a typical example involving an enzymatic reaction consider binding and subsequent hydrolysis of ATP in a solution containing ATP, ADP and P$_i$, at chemical potentials $\mu_T, \mu_D,$ and $\mu_P$, respectively. The enzyme undergoes a transition

$$k + ATP ⇔ (m, ATP) ⇔ (n, ADP, P_i) ⇔ k + ADP + P_i.$$  (29)

We have made explicit that in state $m$ an ATP and that in state $n$ an ADP and a $P_i$ are tightly bound to the enzyme. The overall reaction becomes

$$k + ATP ⇔ k + ADP + P_i.$$  (30)

For simplicity, we have constrained the enzyme to be in the same state $k$ (without bound molecules) before and after the reaction. The free energy difference for the first step involving the binding of the ATP becomes

$$F_2 - F_1 = (F_m^{enz} - \mu_T) - F_m^{enz},$$  (31)

where we use (21) since state $m$ has an ATP bound to it. Likewise, the free energy difference upon release of ADP and $P_i$ becomes

$$F_4 - F_3 = F_k^{enz} - (F_n^{enz} - \mu_D - \mu_P).$$  (32)

Since the overall free energy difference clearly is

$$\Delta F = F_4 - F_1 = \mu_D + \mu_P - \mu_T,$$

(33)

we obtain for the free energy difference between the two intermediate states the expression

$$F_3 - F_2 = (F_3 - F_4) + (F_4 - F_1) + (F_1 - F_2) = F_m^{enz} - F_m^{enz}.$$  (34)

The last line shows the advantage of expressing the free energy of the enzyme in terms of definitions (21). Clearly, the free energy difference between these two intermediate states should not strongly depend on concentrations of the solutes, i.e., should not contain terms that depend explicitly on their chemical potentials.

### 3.3 General case

For a general case, consider transitions typically written as

$$n^- + \sum_i r_i^p A_i ⇔ n^+ + \sum_i s_i^p A_i$$

(35)

where $1 ≤ p ≤ N_p$ labels the possible transitions. Here, $n^-_p$ and $n^+_p$ denote the states of the enzyme before and
after the reaction, respectively. Following the scheme just applied to the example above, we obtain for the free energy difference involved in this transition

$$\Delta F_\rho \equiv \Delta F^\text{enz}_\rho + \Delta F^\text{sol}_\rho$$

(36)

where

$$\Delta F^\text{enz}_\rho \equiv \frac{E^\text{enz}}{n^\rho_\rho} - \frac{E^\text{enz}}{n^\rho_\rho}$$

(37)

denotes the free energy change of the enzyme and

$$\Delta F^\text{sol}_\rho = \sum_i (s^\rho_i - r^\rho_i) \mu_i \equiv \Delta \mu_\rho$$

(38)

denotes the free energy change attributed to the solution in this reaction. Note that these relations remain true even if the states $n^\rho_\rho$ and $n^\rho_\rho$ have both the same additional molecules not showing up in (35) bound to them provided one then uses for their free energies the definition analogously to (21). Likewise, the change in internal energy and entropy of the combined system becomes

$$\Delta E_\rho \equiv \Delta E^\text{enz}_\rho + \Delta E^\text{sol}_\rho$$

(39)

$$= \frac{E^\text{enz}}{n^\rho_\rho} - \frac{E^\text{enz}}{n^\rho_\rho} + \Delta \mu_\rho - T \partial_T \Delta \mu_\rho,$$

(40)

and

$$\Delta S_\rho \equiv \Delta S^\text{enz}_\rho + \Delta S^\text{sol}_\rho$$

(41)

$$= \frac{S^\text{enz}}{n^\rho_\rho} - \frac{S^\text{enz}}{n^\rho_\rho} - \partial_T \Delta \mu_\rho,$$

(42)

respectively.

### 3.4 First law

As in the case of pure conformational changes, we now want to assign a first law type energy balance to each reaction of type $\rho$ shown in (35). Once an initial state is prepared, in the closed system (enzyme plus solution) there is obviously no source of external work. Neither does the system perform any work. Hence, the heat released in this transition is given by minus the change of internal energy of the combined system (39)

$$q_\rho = -\Delta E_\rho = -\Delta E^\text{enz}_\rho - \Delta \mu_\rho + T \partial_T \Delta \mu_\rho.$$  

(43)

This relation shows that the enzyme and the solution are treated on the same footing since only their combined change in internal energy enters. Clearly, since the heat is released into the solution acting as a thermal bath, the configurational change of the enzyme as well as binding and releasing solute molecules contribute to the same bath.

### 4 Molecular motors

#### 4.1 First law

Essentially the same formalism applies to an enzyme acting as a molecular motor often described by such discrete states. Most generally, if the motor undergoes a forward transition of type $\rho$ as in (35), it may advance a distance $d_\rho$ in the direction of the applied force $f$ (or, if $f < 0$, opposite to it). We allow the special cases $d_\rho = 0$ (pure chemical step) or $s^\rho_i = r^\rho_i = 0$ (pure mechanical step) but do not exclude that both types are involved in one transition. For $d_\rho \neq 0$, the mechanical work

$$u^\text{mech}_\rho \equiv fd_\rho$$

(44)

is applied to (or, if negative, delivered by) the motor.

We first consider the case that the motor is operating in an environment where the concentration of molecules like ATP, ADP or P$_i$ are initially fixed. Effectively, these conditions correspond to a closed system as discussed above for an enzymatic reaction. In an almost trivial extension of (43) the first law for a single transition of type $\rho$ becomes

$$q_\rho = u^\text{mech}_\rho - \Delta E_\rho = fd_\rho - \Delta E^\text{enz}_\rho - \Delta \mu_\rho + T \partial_T \Delta \mu_\rho.$$  

(45)

#### 4.2 Comparison to previous work: “Chemiostats”

The form (45) of the first law with the concomitant identification of the heat dissipated in such a transition is original to the present work. It differs from the form discussed previously for molecular motors by Baker [31], and, more recently, in particular by Lipowsky and co-workers [34,35,39,40]. In their work, the first law for a step like in (35) is formulated (using our notation and sign convention) as

$$q_\rho = u^\text{chem}_\rho - \Delta E^\text{enz}_\rho - \Delta \mu_\rho + T \partial_T \Delta \mu_\rho,$$

(46)

where we use the overbar to distinguish their heat

$$q_\rho = q_\rho - T \partial_T \Delta \mu_\rho = q_\rho + T \Delta S^\text{sol}_\rho,$$

(47)

from the present $q_\rho$. If the heat released in one step is a physically meaningful concept, it should be unique. Hence, only one expression, either $q_\rho$ or $\tilde{q}_\rho$, can be the correct one.

Formally, the two expressions for the heat differ by a term involving the entropy change in the solution resulting from the reaction. The physical origin of the two different forms arises from the fact that in the previous work the enzyme is thought to be coupled to “chemiostats” providing and accepting molecules at an energetic cost (or benefit) given by their chemical potential. Introducing the notion of a chemical work

$$w^\text{chem}_\rho \equiv -\Delta \mu_\rho$$

(48)

the first law is then written in the form

$$w^\text{mech}_\rho + w^\text{chem}_\rho = \Delta E^\text{enz}_\rho + \tilde{q}_\rho.$$  

(49)

The origin of the difference between the two approaches becomes clear by analyzing the operation of chemiostats in more detail in the context of whether the concentration of the $A_i$ molecules are kept strictly constant or not. This distinction has been alluded to in our previous work on enzymatic reactions [13] and biochemical reaction networks.
where the same subtlety arises but it seems appropriate to provide a more explicit and detailed discussion in order to settle this important point. Essentially, one has to distinguish two different scenarios.

Scenario I is the one discussed in the present paper so far where we prepare a non-equilibrium state by selecting non-equilibrium concentrations \( \{c_i\} \) and then let the motor run. The first law in the form \( \sum E_{\text{sol}}^\text{re} \) and the corresponding identification of the heat then seems inevitable. A side effect of such a set-up, however, is the fact that strictly speaking the concentrations \( \{c_i\} \) will (slowly) change in a finite system. Insisting on a strictly constant concentration will lead us to scenario II.

In this second scenario, one wants to control the concentrations of these solute molecules throughout the experiment. Literally speaking, one then has to refill or extract certain molecules after a reaction event has taken place. Practically, this can obviously not be done in any strict manner. Conceptually, however, we can conceive devices, which are effectively the chemiostats, that “reset” the number of solute molecules after each step. Of course, such an intervention has to obey a first law as well which we formulate for such a reset operation following the reaction of type \( \rho \) as

\[
w_{\rho}^{\text{re}} = \Delta E_{\rho}^{\text{sol,} \text{re}} + q_{\rho}^{\text{re}} = -\Delta E_{\rho}^{\text{sol}} + q_{\rho}^{\text{re}},
\]

where the superscript “re” stands for reset. The change in internal energy of the solution \( \Delta E_{\rho}^{\text{sol,} \text{re}} \) is minus the corresponding change in internal energy of the solution, i.e., \( -\Delta E_{\rho}^{\text{sol}} \), when the reaction took place. If this reset operation occurs quasistatically, the work \( w_{\rho}^{\text{re}} \) spent in it is equal to the free energy change of the solution in this operation, which is \( -\Delta \mu_{\rho} \), leading to the identification

\[
q_{\rho}^{\text{re}} = -\Delta \mu_{\rho} + \Delta E_{\rho}^{\text{sol}} = T \Delta S_{\rho}^{\text{sol}} = q_{\rho} - q_{\rho}.
\]

Hence, the heat \( q_{\rho} \) discussed in previous works for a single step under chemiostated conditions physically would correspond to the sum of (i) the heat \( q_{\rho} \) dissipated in the reaction step and (ii) the heat \( q_{\rho}^{\text{re}} \) dissipated in the subsequent quasistatic steps when the molecules involved in the reaction are fed in and taken out. Note that such a procedure makes sure that the concentrations literally have not changed at all.

While this scenario II may be possible conceptually it is difficult to envisage a practical experimental implementation. It thus seems that for identifying the heat the approach taken in the present paper is the physically more realistic and relevant one since the motor accepts and releases molecules directly from the surrounding solution with no further feed-back-type interference from chemiostats.

5 Entropy production

5.1 Motivation

Naively, one might have expected that with the correct identification of both the heat, which should be attributed to a change of the entropy of the surrounding heat bath, or ”medium”, via

\[
\Delta S_{\rho}^{\text{med}} \equiv q_{\rho}/T,
\]

and the intrinsic entropy change of the system \( \Delta S_{\rho} \) as given by \( (11) \) the total entropy change in one step is given by the sum of both, i.e., \( \Delta S = \Delta S_{\rho}^{\text{med}} + \Delta S_{\rho} \).

For a counterexample showing that such a view would be too simplistic consider an enzyme with just two states, \( m \) and \( n \), with \( E_{mn}^{\text{enz}} = E_{nm}^{\text{enz}} \) and \( S_{mn}^{\text{enz}} > S_{nm}^{\text{enz}} \). If the enzyme is initially in state \( m \) it will at some time jump to state \( n \). Such an isoenergetic transition involves no exchanged heat and hence no change in the entropy of the medium. Clearly, then the sum of the changes in intrinsic system entropy and medium entropy is negative for such a transition. While this is not a problem for a single enzyme it becomes one if we consider a whole ensemble of enzymes all prepared initially in state \( m \). Likewise, we could repeat the experiment with a single enzyme initially prepared in state \( m \) many times for obtaining an ensemble average. In both cases, naively averaging the total entropy change as tentatively identified above, we would get on average a decrease in total entropy. Such a conclusion violates the second law and hence something is missing. We now show that we have to add a third contribution to the entropy, called stochastic entropy \( [7] \), in order to achieve a consistent description of entropy changes on the level of transitions along an individual trajectory. This concept necessarily requires an ensemble description from which the individual trajectories are taken.

On the ensemble level, entropy production in (bio)chemical reaction networks has been investigated for quite some time using master equation \([10, 11, 12, 13, 14]\). The main point in the following will not be to repeat this analysis but rather to use consistency with the second law on the ensemble level together with the insight into the first law derived above for a complete identification of the entropy production to be associated with an individual transition on the trajectory level.

5.2 Ensemble

In the course of time, the enzyme will jump between different states. The jump times will be stochastic since there is only a certain probability that a reaction of type \( (35) \) takes place if the enzyme is in the state \( n_{\rho} \) corresponding to the left hand side of \( (35) \). A trajectory of the enzyme can then be characterized by the sequence of jump times \( \{t_j\} \) and the sequence of reactions \( \{\rho_j\} \) where \( \rho_j \) denotes the corresponding reactions and \( \sigma_j = \pm \) characterizes the direction in which the reaction takes place, see Fig. 2 for an example based on the scheme \([20]\).

An ensemble is defined by specifying (i) the initial probability \( p_n(0) \) for finding the enzyme in state \( n \) and (ii) the set of rates \( w_{\rho}^{\pm} \) with which the reactions \( 35 \) takes place in either direction. Both inputs will then determine the probability \( p_n(t) \) to find the enzyme in state \( n \) at time \( t \). Averages with respect to such an ensemble will be denoted by \( \langle \ldots \rangle \).
5.3 Entropy production in one step

As explained above, naively adding the entropy change of the medium and the intrinsic one of the system will not necessarily lead to an on average non-negative entropy production. Therefore, we tentatively write the total entropy change occurring in a forward transition of type \( \rho \)

\[
\Delta S^\text{tot}_\rho(t) = \Delta S^\text{med}_\rho + \Delta S_\rho + \Delta S_\rho(t)
\]

where the last term is the one still to be determined. The reason for introducing an explicit time-dependence will become clear below. The corresponding value of the total entropy change involved in a backward transition would be \(-\Delta S^\text{tot}_\rho(t)\). As an essential requirement, we impose the condition that the average total entropy production rate is non-negative, i.e. that

\[
0 \leq \langle \dot{S}^\text{tot}(t) \rangle = \sum_\rho [p_{n^-\rho}(t) w^+_{n^-\rho} - p_{n^+\rho}(t) w^-_{n^+\rho}] \Delta S^\text{tot}_\rho(t)
\]

where here and in the following the dot denotes a time-derivative, i.e., a rate. The explicit expression for this average arises from exploiting the fact that with probability \( p_{n^-\rho}(t) \) the enzyme is in state \( n^-\rho \) allowing at time \( t \) the reaction \( \rho \) to take place in forward direction with rate \( w^+_{n^-\rho} \). Likewise, the enzyme is with probability \( p_{n^+\rho}(t) \) in the state \( n^+\rho \) allowing the reaction to proceed in backward direction.

Now suppose that we knew the rates \( w^\pm_{n^-\rho} \) and were looking for \( \Delta S^\text{tot}_\rho(t) \) as a function of \( p_{n^-\rho}(t), p_{n^+\rho}(t) \) and these rates. Since the inequality \( \langle \dot{S}^\text{tot}(t) \rangle \geq 0 \) has to be respected for any \( p_n(t) \), it looks inevitable that each individual term in this sum has to be non-negative, i.e.,

\[
0 \leq [p_{n^-\rho} w^+_{n^-\rho} - p_{n^+\rho} w^-_{n^+\rho}] \Delta S^\text{tot}_\rho \equiv (y - x) \Delta S^\text{tot}_\rho (x, y)
\]

which defines the abbreviations \( x \) and \( y \) and where we suppress the \( t \)-dependence notationally. The yet unknown function \( \Delta S^\text{tot}_\rho \) has dimension of entropy, i.e., we can write

\[
\Delta S^\text{tot}_\rho = k_B f(x, y)
\]

Since the function \( f(x, y) \) is dimensionless, it can depend only on a dimensionless variable, i.e. \( f(x, y) = g(y/x) = g(z) \). Finally, the requirement that interchanging forward and backward directions of the reaction \( \rho \), which amounts to interchanging \( x \) and \( y \), corresponds to a sign change in the entropy imposes the condition

\[
g(z) = -g(1/z).
\]

Up to an overall amplitude \( c \), the solution of this functional equation is unique and given by \( g(z) = c \ln z \). Choosing \( c = 1 \) which a posteriori will guarantee consistency with known special cases, we thus obtain the expression

\[
\Delta S^\text{tot}_\rho(t) = k_B \ln \frac{p_{n^-\rho}(t) w^+_{n^-\rho}}{p_{n^+\rho}(t) w^-_{n^+\rho}}
\]

for the total entropy change induced by a forward transition \( \rho \). By separating the time-dependent part from the time-independent one and by comparing with \eqref{eq:53}, we can now identify both (i) the missing piece in the total entropy change in one transition as

\[
\Delta s_\rho(t) = -k_B \ln \frac{p_{n^+\rho}(t)}{p_{n^-\rho}(t)}
\]

and (ii) a consistency relation between the yet unknown rates and the previously defined entropy change of medium and system given by

\[
\Delta S^\text{med}_\rho + \Delta S_\rho = k_B \ln \frac{w^+_{n^-\rho}}{w^-_{n^+\rho}}.
\]

Both identification make sense as we will show in the next two subsections.

5.4 Stochastic entropy

Quite generally, in a time-dependent ensemble specified by probabilities \( p_n(t) \) stochastic entropy of the system has been defined as

\[
s(t) \equiv -k_B \ln p_n(t)
\]

along any individual trajectory \( n(t) \) taken from the specified ensemble. Hence, if a transition of type \( \rho \) takes place at time \( t \), the full entropy change of the system \( \Delta S^\text{sys}_\rho(t) \) consists of the change in stochastic entropy \( \Delta S^\text{sys}_\rho(t) \) and that in intrinsic entropy \( \Delta S_\rho \). Explicitly, one obtains

\[
\Delta S^\text{sys}_\rho(t) \equiv \Delta s_\rho(t) + \Delta S_\rho = -k_B \ln \frac{p_{n^-\rho}(t)}{p_{n^+\rho}(t)} + \Delta S_\rho.
\]
Keeping the time argument is crucial since in a time-dependent ensemble the same transition leads to a different contribution depending on when it takes place.

Finally, adding the concomitant change in entropy of the heat bath $\Delta S^\text{med}_p = q_p/T$, we obtain for the total entropy change associated with this transition the expression \(\Delta S^\text{sys}_p(t) = \Delta S^\text{med}_p + \Delta S^\text{sys}_p(t)\). Note that in an equilibrium ensemble, where global detailed balance applies, i.e., for $p_n(t) = p_n^\text{eq}$ and $p_n^\text{eq} = p_n^\text{eq} w_p^\text{eq} w_p^\text{eq}$, for each jump the contribution to system entropy and medium entropy exactly compensate each other so that the total entropy remains strictly constant along any individual trajectory.

### 5.5 Rates and local detailed balance

The consistency relation derived above in [60] between the ratio of the rates and the sum of medium and system entropy change can be reformulated as

\[
\frac{w_p^+}{w_p^-} = \exp[-\beta \Delta F_p] = \exp[-\beta(\Delta F_p^{\text{enz}} + \Delta \mu_p)],
\]

(63)

for the case of an enzymatic reaction and as

\[
\frac{w_p^+}{w_p^-} = \exp[-\beta(\Delta F_p^{\text{enz}} + \Delta \mu_p - F_{\text{mech}})],
\]

(64)

in the case of a motor protein where this transition involves external work, respectively. Here, we have used \[64\] and the first law in the form \[13\] and \[15\], respectively. For molecular motors, the additional exponential factors express the contribution of an applied force. In both cases, all quantities depend on the concentrations \{c\).

Both relations for the ratio of the rates of forward reaction to backward reaction are well known under the notion of “local detailed balance”. In the present work, we have shown that the rates have to obey this relation in order to get positive total entropy production in a time-dependent ensemble.

### 5.6 Dynamical formulation of the first law

It is instructive to reformulate the two variants of the first law discussed above for molecular motors in terms of these rates. For a closed system, prepared with non-equilibrium conditions, the heat released in this transition becomes

\[
\dot{q}_p = T \left( k_B \ln \frac{w_p^+}{w_p^-} - \Delta S_p \right),
\]

(65)

irrespective of whether external mechanical work is involved or not. This expression shows that due to the presence of the intrinsic entropy change $\Delta S_p$, the heat dissipated in one transition cannot be inferred by just measuring the ratio of the rates.

For the alternative case of chemostats with the explicit refreeing and taking out of used and produced solutes one gets

\[
\dot{q}_p = T \left( k_B \ln \frac{w_p^+}{w_p^-} - \Delta S_p^{\text{enz}} \right).
\]

(66)

For molecular motors often the case of a full cycle is discussed after which the enzyme comes back to its initial internal state. Note that for the scenario involving the chemostats, the heat $\dot{q}$ dissipated along the cycle can then be expressed by just the logarithm of the ratio of the product of forward and backward rates along the cycle. For the physically more realistic heat $q$, one has to correct for the entropy change in the solution in order to determine the heat from the product of the rates along a cycle.

### 5.7 Fluctuation theorems

So far, we have analyzed the changes in thermodynamic quantities caused by an individual transition. By summing up the contributions from all transitions $\rho_j^\text{eq}$ happening during a time interval $t_i < t < t_f$ and taking into account a possible change in stochastic entropy $s(t)$ due to an explicit time-dependence in $p_n(t)$ while the system stays in one state, one obtains the total entropy change along a trajectory during this time interval as

\[
\Delta S^\text{tot} = k_B \sum_j \ln \frac{w_p^+}{w_p^-} + s(t_f) - s(t_i).
\]

(67)

This quantity obeys a relation called the integral fluctuation theorem for entropy production [9]

\[
\langle \exp[-\Delta S^\text{tot}/k_B] \rangle = 1,
\]

(68)

where the average $\langle ... \rangle$ is over many trajectories taken from any well-defined initial ensemble characterized by $p_n(t_i)$ and running for an arbitrary but fixed time interval $t_f - t_i$. From this integral relation one gets easily the second-law like statement on the mean total entropy production

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

(69)

In the approach promoted in this paper, rather than “deriving” the latter relation from the integral fluctuation theorem, we have used it as an essential consistency requirement for (i) arguing that stochastic entropy is a crucial contribution to the entropy change occurring in an individual transition and (ii) showing that rates obeying the local detailed balance relations \[63,64\] are required by thermodynamic consistency.

For a non-equilibrium steady state where $p_n(t) = p_n$ is independent of time, one has the detailed fluctuation theorem

\[
p(-\Delta S^\text{tot})/p(\Delta S^\text{tot}) = \exp(-\Delta S^\text{tot}/k_B)
\]

(70)

for the probability distribution $p(\Delta S^\text{tot})$ to observe a certain total entropy production valid for any time interval in this non-equilibrium steady state [9].
6 Conclusions

The present analysis is supposed to reveal more clearly than previous work both the structural coherence and some finer issues of the stochastic thermodynamics of single enzymes and molecular motors. We first summarize the main assumptions and consequences of this approach.

On the state level, internal energy, intrinsic entropy and free energy follow from an underlying microscopic model if one assumes a time-scale separation between transitions within each state and the slower and observable transitions between these states. It is not necessary to assume weak interactions between enzyme and solutes. The first law with a concomitant identification of heat dissipated in one transition then follows almost trivially. This form of the first law is in disagreement with formulations for motor proteins where chemiostats have been invoked as source of “chemical work”. We have argued that their application is time-dependent as well. In consequence, both the quantities appearing in the first law and entropy production can pick up contributions even while the enzyme remains in the same state.

Finally, with the conceptual basis thus solidified, these thermodynamic notions should now be applied to data from single enzyme experiments. A very promising molecule seems to the F1-ATPase for which the first experimental studies using such concepts have just appeared.

7 Appendix: Non-equilibrium ensemble thermodynamics

The main intention of the approach discussed in this paper has been the identification of thermodynamic quantities not on the ensemble level but for a single enzyme along its fluctuating trajectory taken from a well-defined ensemble. For completeness and future reference, we briefly present the consequences of our thermodynamically consistent approach for time-dependent averages.

Quite generally, once for each state \( n \) of a system an internal energy \( E_n \), an intrinsic entropy \( S_n \), and a free energy \( F_n \) are identified from a more microscopic model, the ensemble average of the internal energy becomes

\[
\langle E \rangle \equiv \langle E(t) \rangle = \sum_n p_n(t) E_n. \tag{71}
\]

The appropriate ensemble averaged entropy of the system is given by

\[
\langle S \rangle \equiv \sum_n p_n(t) [S_n + s_n(t)], \tag{72}
\]

with \( s_n(t) \equiv -k_B \ln p_n(t) \). A “dynamical free energy” on the ensemble level then follows from the usual thermodynamic relation as

\[
\langle F \rangle \equiv \langle E \rangle - \langle TS \rangle = \sum_n p_n(t) [F_n - T s_n(t)]. \tag{73}
\]

Note that both quantities, \( \langle S \rangle \) and \( \langle F \rangle \), cannot be obtained by simply averaging over the state variable \( S_n \) or \( F_n \) as it is possible for the internal energy. The expression \( s_n(t) \) arising from stochastic entropy in the square brackets in (72) and (73) is rather ensemble dependent and thus not a genuine state variable.

For a consistency check of these ensemble expressions, consider an equilibrium ensemble. Then the probability to find the system in any microstate \( \xi \) is given by

\[
p^\text{eq}(\xi) = \exp[-\beta(V^{\text{tot}}(\xi) - \mathcal{F}^\text{eq})]. \tag{74}
\]
with the equilibrium free energy
\[ F_{\text{eq}} = -k_B T \ln \sum_\xi \exp[-\beta V^\text{tot}(\xi)]. \] (75)
for the total system consisting of enzyme including the surrounding solution. Likewise, one has for the ensemble internal energy
\[ E_{\text{eq}} = \sum_\xi p^\text{eq}_\xi V^\text{tot}(\xi) \] (76)
and the ensemble system entropy
\[ S_{\text{eq}} = -k_B \sum_\xi p^\text{eq}_\xi \ln p^\text{eq}_\xi = (E_{\text{eq}} - F_{\text{eq}})/T. \] (77)

It is easily checked that the time-dependent quantities \[71\,73\] agree with these equilibrium ensemble quantities, if \( p_n(t) \) in \[71\,73\] becomes the equilibrium probability
\[ p^\text{eq}_n \equiv \exp[-\beta(F_n - F_{\text{eq}})]. \] (78)
with \( F_n \) as previously defined in \[83\].

For the rate of change of the internal energy of the system, i.e., here the enzyme or motor plus the surrounding solution, one obtains
\[ \dot{E}(t) = \sum_\rho (p^+_{n\rho}(t)w^+_{\rho} - p^-_{n\rho}(t)w^-_{\rho}) \Delta E_{\rho}. \] (79)
This expression enters the rate formulation of the first law on the ensemble level as given by
\[ \dot{W}^{\text{mech}}(t) = \dot{Q}(t) + \dot{E}(t) \] (80)
with
\[ \dot{W}^{\text{mech}}(t) = \sum_\rho (p^-_{n\rho}(t)w^+_{\rho} - p^+_{n\rho}(t)w^-_{\rho}) w^{\text{mech}}_{\rho}. \] (81)
as the ensemble averaged work rate. The heat dissipation rate on the ensemble level thus becomes
\[ \dot{Q}(t) = \sum_\rho (p^+_{n\rho}(t)w^+_{\rho} - p^-_{n\rho}(t)w^-_{\rho}) q_{\rho}. \] (82)
Likewise, for the full entropy change of the system, one obtains
\[ \dot{S}(t) = \sum_\rho (p^+_{n\rho}(t)w^+_{\rho} - p^-_{n\rho}(t)w^-_{\rho}) \Delta S^\text{sys}_\rho(t), \] (83)
with \( \Delta S^\text{sys}_\rho(t) \) from \[82\] and for the corresponding change in free energy
\[ \dot{F}(t) = \dot{E}(t) - T \dot{S}(t) \] (84)
\[ = \sum_\rho (p^+_{n\rho}(t)w^+_{\rho} - p^-_{n\rho}(t)w^-_{\rho}) \times \] (85)
\[ \times (\Delta E_{\rho} - T \Delta S^\text{sys}_\rho(t)). \] (86)
Note that if no external work is applied, the thus defined free energy dissipation rate is exactly given by \((-T)\) times the total entropy production rate \[\dot{\Sigma}\].

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