Stochastic thermodynamics of chemical reaction networks

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For chemical reaction networks in a dilute solution described by a master equation, we define energy and entropy on a stochastic trajectory and develop a consistent nonequilibrium thermodynamic description along a single stochastic trajectory of reaction events. A first-law like energy balance relates internal energy, applied (chemical) work and dissipated heat for every single reaction. Entropy production along a single trajectory involves a sum over changes in the entropy of the network itself and the entropy of the medium. The latter is given by the exchanged heat identified through the first law. Total entropy production is constrained by an integral fluctuation theorem for networks arbitrarily driven by time-dependent rates and a detailed fluctuation theorem for networks in the steady state. Further exact relations like a generalized Jarzynski relation and a generalized Clausius inequality are discussed. We illustrate these results for a three-species cyclic reaction network which exhibits nonequilibrium steady states as well as transitions between different steady states.

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I. INTRODUCTION

Networks of coupled chemical reactions in a dilute solution should be described by a chemical master equation whenever fluctuations are relevant due to small numbers of at least one of the involved species. As essential parameters this equation contains the rate constants of all possible reactions. The solution of the chemical master equation gives the dynamics of the probability to find a certain number of molecules of each species at a given time for a given initial condition. The stochastic character of such a description implies that for each realization we can talk about the stochastic trajectory of the network by recording, in principle, the time at which each particular reaction took place with its concomitant change of the number of molecules. Even though such a description can be applied to networks in thermodynamic equilibrium, it is particularly relevant for driven networks in open systems where externally imposed boundary conditions generate fluxes even at a constant (mean) concentration of species. More generally, such a description holds true even if the rate “constants” become time-dependent, e.g., if the concentration of a chemical species can be controlled externally in a time-dependent fashion.

Quite often, such networks are effectively at constant temperature and pressure, i.e., at thermodynamically well-defined conditions. This holds true in particular for the small networks discussed in cell biology where the few relevant molecules are embedded in the aqueous intracellular solution. Such networks describing, e.g., gene regulation, signal transduction (see, e.g., molecular motors, operate typically in non-equilibrium generated by (time-dependent) external mechanical or chemical stimuli. Feedback loops can generate cycles as a characteristics of non-equilibrium steady states. Taking both the fluctuation aspects and the well-defined thermodynamic conditions seriously, the question arises whether or not it is possible to give a thermodynamically consistent description of such networks on the level of a single (stochastic) trajectory.

Thermodynamics on the level of a fluctuating trajectory in a driven system may look far fetched at first sight. Such an approach, however, has been extremely fruitful for somewhat different but conceptually related systems consisting of a few degrees of freedom, like colloidal particles or (bio)polymers. The dynamics of these systems in aqueous solution under the action of external forces generated mechanically by the tip of an atomic force microscope or optically by laser tweezers can be typically described by a Langevin equation. For such a driven dynamics, a thermodynamically consistent description has recently been worked out based on two essential ingredients which can also be seen as the essential assumptions of such an approach. (i) A first-law like balance relates external work, internal energy and exchanged heat dynamically along each fluctuating trajectory. (ii) The heat dissipated into the environment leads to a well-defined entropy increase of the surrounding medium which is assumed to keep locally its constant temperature despite the fact that the few degrees of freedom of the system (colloidal particle or polymer) embedded into this bath are driven to nonequilibrium. For such systems, various exact relations have been derived theoretically. The most prominent examples are the Jarzynski relation which allows to extract free energy differences from experiment or simulations performed under non-equilibrium conditions and the fluctuation theorem which quantifies the probability to observe entropy annihilating trajectories in the steady state. These relations and various ramifications have been tested in real and computer experiments both using mechanical stretching of biomolecules as well as colloidal particles driven by laser traps. Since the fundamental constituents (a stochastic trajectory, a surrounding heat bath and some source of non-equilibrium) of these mechanical systems are also present in chemical networks as described above, a similar approach should be feasible for them as well. The purpose of this paper is to develop this correspondence and explore its consequences in some
Fluctuation theorems have indeed been discussed previously both for the steady state of non-equilibrium networks by Gaspard and coworkers and for time-dependently driven chemical reactions in Ref. 44 without making the connection to a first-law energy balance. Qian and co-workers have developed such an energetic perspective mainly on the ensemble level extending Hill’s classical works. On the trajectory level, unpublished work by Shibata 45 and our previous studies on simple models for enzymes point in this direction.

The present systematic approach towards a stochastic thermodynamics for chemical networks based on a master equation rests on two main ingredients. First, we apply the first law to a single trajectory, i.e. we analyse a single reaction event in terms of chemical work applied by the chemiostats, change in internal energy and amount of dissipated heat. This allows us to express the exchanged heat in each reaction step in terms of the rate constants of the master equation. Second, we define entropy production also along a single trajectory as a sum of two contributions. The heat dissipated in each step increases the entropy of the surrounding medium. Moreover, there is an entropy of the network itself. The sum of both changes obeys various exact relations from which, e.g., the second law follows trivially for the mean entropy production.

The paper is organized as follows. In Section II we recall the stochastic modelling of chemical reaction networks by a master equation approach. In Section III we define energy, work and dissipated heat on a stochastic trajectory and develop a consistent nonequilibrium first law along a single stochastic trajectory of reaction events. In Section IV we define stochastic entropy along a trajectory of the system and total entropy production of system and heat bath. In Section V we derive a fluctuation theorem for the total entropy production from which a second-law like statement follows directly. We then relate this fluctuation theorem to the Jarzynski relation. The total entropy production can be split up into a housekeeping heat, which accounts for the irreversibility due to nonequilibrium steady states, and a quantity, which accounts for the irreversibility due to transitions between steady states. Both quantities obey a generalized fluctuation theorem. In Section VI we apply our results to a paradigmatic reaction network.

II. MODEL

A. Chemical master equation

We consider the general reaction network

\[
\sum_{\alpha=1}^{N_\alpha} r^\alpha \, A_\alpha + \sum_{j=1}^{N_j} p_j^\alpha \, X_j = \sum_{\alpha=1}^{N_\alpha} s^\alpha \, A_\alpha + \sum_{j=1}^{N_j} q_j^\alpha \, X_j
\]  

where \( r^\alpha \) and \( s^\alpha \) are the forward and reverse rate constants, \( p_j^\alpha \) and \( q_j^\alpha \) denote the forward and backward chemical reaction rates, \( N_\alpha \) and \( N_j \) are the numbers of species of type \( \alpha \) and reactions, respectively. The vector notation is used for clarity.

FIG. 1: Coupling of the system with species \( X_j, j = (1, \ldots, N_j) \) to the \( N_\alpha \) particle reservoirs for species \( A_\alpha \) at chemical potential \( \mu_\alpha \) and to a heat bath at constant temperature \( T \).

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\]  

with \( 1 \leq r \leq N_\alpha \) labeling the single (reversible) reactions. We distinguish two types of reacting species. The \( X_j \) molecules \( j = (1, \ldots, N_j) \) are those species whose numbers \( n = (n_1, \ldots, n_{N_j}) \) can, in principle, be measured along a chain of reaction events. In practice, these numbers should be small. The \( A_\alpha \) molecules \( \alpha = (1, \ldots, N_\alpha) \) correspond to those species whose overall concentrations \( c_\alpha \) are controlled externally by a chemiostat due to a (generally) time dependent protocol \( c_\alpha(t) \). In principle, this implies that after a reaction event has taken place, the used \( A_\alpha \) are “refilled” and the produced ones are “extracted”. These chemiostats have chemical potential

\[
\mu_\alpha = \mu^0_\alpha + T \ln \left( \frac{c_\alpha}{c_0} \right)
\]  

where \( \mu^0_\alpha \) is the chemical potential for standard conditions at concentration \( c_0 \) and \( T \) is the temperature of the heat bath to which both type of particles are coupled, see Fig. 1. Throughout the paper we set Boltzmann’s constant to unity which implies that entropy will be dimensionless. We assume that the reacting species have no internal degrees of freedom. However, internal degrees of freedom could easily be treated within our approach by labeling different internal states as different species and defining “reactions” (transitions) between them.

The stochiometric coefficients \( r_\alpha, p_j, s_\alpha \) and \( q_j \) enter the stochiometric matrix \( V \) with entries

\[
v_j^\alpha = q_j^\alpha - p_j^\alpha
\]  

and the stochiometric matrix of the reservoir species \( U \) with entries

\[
u_j^\alpha = s_j^\alpha - r_j^\alpha.
\]  

For the externally controlled concentrations \( c_\alpha \) of \( A_\alpha \), we will use the vector notation \( c = (c_1, \ldots, c_{N_\alpha}) \) in the following. We assume a dilute solution of reacting species in
a solvent (heat bath) and therefore the transition probabilities per unit time for the \( N_p \) reactions take the text book form:

\[
wp^\rho(n, c) = \Omega k^\rho_+ \prod_\alpha (c_\alpha \omega_\alpha)^{\alpha^\rho} \cdot \frac{n_j!}{(n_j - p^\rho_j)! \cdot (\Omega/\omega_j)^{p^\rho_j}} \tag{5}
\]

where + denotes a forward reaction, − denotes a backward reaction and \( \Omega \) is the reaction volume. The bare rates \( k^\rho_{+,-} \) are the transition probabilities per unit time for the \( + \) and \( - \) reactions (1) take the microscopic Hamiltonian.

Using (5) and (6), we get

\[
wp^\rho(n, c) = \Omega k^\rho_+ \prod_\alpha (c_\alpha \omega_\alpha)^{\alpha^\rho} \cdot \frac{n_j!}{(n_j - p^\rho_j)! \cdot (\Omega/\omega_j)^{p^\rho_j}} \tag{6}
\]

Inserting a multivariate Poissonian

\[
p^{eq}(n, c) = \prod_j \frac{1}{n_j!} (n_j^s)^{n_j} e^{-n_j^s} \tag{10}
\]

with mean \( n_j^s \) into equation (10) yields the \( N_p \) equations:

\[
\frac{k^\rho_+}{k^\rho_-} = \prod_\alpha (c_\alpha \omega_\alpha)^{\alpha^\rho} \prod_j \frac{(n_j^s \omega_j)^{p^\rho_j}}{(\Omega)^{p^\rho_j}}. \tag{11}
\]

These \( N_p \) equations are equivalent to the system of linear equations

\[
\sum_j w^\rho_j v_j = \ln \frac{k^\rho_+}{k^\rho_-} + \sum_\alpha w^\rho_\alpha \ln (c_\alpha \omega_\alpha) \tag{12}
\]

with

\[

\epsilon_j \equiv - \ln (n_j^s \omega_j / \Omega). \tag{13}
\]

We now have to distinguish three cases concerning the solvability of the system which lead to three different physical situations.

**Case I : Equations have a unique solution.**

Generically, this case requires \( N_p > N_j \). The unique solution of eqs. (12) corresponds to the unique equilibrium distribution, if all states are accessible from every initial state through a sequence of reaction events. The equilibrium distribution (14) can then be written as

\[
p^{eq}(n, c) = N \prod_j \frac{1}{n_j!} (\Omega/\omega_j)^{n_j} e^{-\epsilon_j, n_j} \tag{14}
\]

with normalization factor \( N = \prod_j \omega_j^{n_j} \). Taken at face value, the form (14) resembles a grandcanonical distribution with one particle energies \( \epsilon_j \) and chemical potentials \( \mu_j = 0 \). As we will show below, consistency requires splitting \( \epsilon_j \) in a one-particle energy \( E_j \) and a non-zero chemical potential \( \mu_j \).

However, even if eqs. (12) have a unique solution, not all states must necessarily be accessible from every initial state through a sequence of reaction events due to the discrete reaction kinetics. The equilibrium distribution then is the projection of the Poissonian (14) to the subspace of accessible states.

**Case II : Equations have more than one solution.**

The rank of the stochiometric matrix \( V \) then is smaller than the number \( N_j \) of species \( X_j \). This is usually the case if there are less reactions \( \rho \) than species \( X_j \), i.e. \( N_p < N_j \). We then have constraints for the accessible state space depending on the initial distribution. The
state space \( \{ \mathbf{n} \} \) is then separated into an infinite number of subspaces. If all possible initial states lie in the same subspace, the equilibrium distribution is the projection of the Poissonian to this subspace with the \( \epsilon_j \) given by any solution of \( \{ \mathbf{12} \} \). As is shown in App. \( \mathbf{A} \), it does not matter which solution of \( \{ \mathbf{12} \} \) is chosen.

**Case III : Equations \( \{ \mathbf{12} \} \) have no solution.**

The chosen \( c_\alpha \) then create a genuine nonequilibrium stationary state (NESS) violating the detailed balance condition. This will usually happen, if there are more reactions \( \rho \) than species \( X_j \), i.e., \( N_\rho > N_J \). We then have essentially different paths leading to the same final state, i.e., there are genuine cycles in the network.

Even if the network has no equilibrium solution for the given \( c_\alpha \), there must be certain values \( c_\alpha^\text{eq} \) for which eqs. \( \{ \mathbf{12} \} \) have a solution. For we can always assume to isolate the system after preparing an initial state with given \( c_\alpha \). The network will then relax into an equilibrium state with concentrations \( c_\alpha^\text{eq} \).

**III. FIRST LAW**

We want to state the first law of thermodynamics along a stochastic trajectory \( \mathbf{n}(\tau) \) for an arbitrary network \( \mathbf{11} \). We therefore need the concept of internal energy, chemical work and dissipated heat.

**A. Internal energy**

We start with internal energy and consider an equilibrium situation with concentrations \( c_\alpha^\text{eq} \) which are even defined for case III, as discussed above. Then, a solution of \( \{ \mathbf{12} \} \) exists for this set of reservoir concentrations \( c_\alpha^\text{eq} \). We also assume a microscopic Hamiltonian for the dilute solution of \( X_j \) and \( A_\alpha \) molecules in the solvent from which an (average) energy \( E_j \) and \( E_\alpha \) for the reacting species \( X_j \) and \( A_\alpha \), respectively, can be derived as shown in App. \( \mathbf{A} \). The chemical potential \( \mu_\alpha \) then can be written as

\[
\mu_\alpha = E_\alpha + T \ln (c_\alpha \omega_\alpha) \tag{15}
\]

where the volumes \( \omega_\alpha \) are chosen such that \( E_\alpha \) becomes the energy of one molecule \( A_\alpha \) as defined by a microscopic Hamiltonian, see App. \( \mathbf{A} \) for details. If, for example, the \( A_\alpha \) could be treated as an ideal gas without internal degrees of freedom, this normalization volume would become \( \omega_\alpha \equiv \lambda_\alpha^3 \exp(-3/2) \) where \( \lambda_\alpha \) is the thermal de Broglie wavelength of an \( A_\alpha \) molecule and the factor \( \exp(-3/2) \) guarantees that the thermal kinetic energy is included in the energy \( E_\alpha \) of an \( A_\alpha \) molecule.

Even though the rewriting from eq. \( \{ \mathbf{2} \} \) to eq. \( \{ \mathbf{15} \} \) looks trivial, it is a crucial step since it provides the relation between the energy \( E_\alpha \) which will enter the first law and the chemical potential \( \mu_\alpha \). The very fact that there is a concentration \( c_\alpha = 1/\omega_\alpha \) where both become numerically identical does, of course, not imply that these two quantities are similar in general. In fact, the volume factor \( \omega_\alpha \) as derived in App. \( \mathbf{A} \) depends on the microscopic details.

The key point for the subsequent analysis is that the energies \( E_j \) thus identified in an equilibrium situation from statistical mechanics are independent from \( e^{\mathbf{eq}} \). Since in a dilute solution, there is no interaction between the reacting species apart from the chemical reactions, the energy \( E_j \) of one molecule \( X_j \) will neither depend on the number of molecules \( n_i \) of the other species nor on the concentrations \( c_\alpha \). Hence, the \( E_j \) are well defined even for networks in a NESS and for networks driven by time-dependent \( c_\alpha (\tau) \). Likewise, the definition of the chemical potential of reservoir species \( \mu_\alpha \) still holds true in such nonequilibrium situations, since the particle reservoirs are assumed to be large.

A system energy or internal energy

\[
E(\mathbf{n}(\tau)) = \sum_j E_j n_j(\tau) \tag{16}
\]

along a stochastic trajectory can then be defined both in equilibrium and under the specified non-equilibrium conditions.

**B. Work and heat**

For a formulation of the first law along a stochastic trajectory, we next have to calculate the heat flowing from the system into the heat bath. Whenever a reaction \( \rho \) takes place in forward direction \( (r = +1) \) or backward direction \( (r = -1) \), i.e., whenever \( \mathbf{n} \) changes, the energy of the system changes by

\[
\Delta E^{\pm,\rho} \equiv \Delta E^{\pm,\rho}_\text{chem} \equiv \sum_j E_j \Delta n_j^{\pm,\rho} = \pm \sum_j E_j v_j^\rho. \tag{17}
\]

where \( \Delta n_j^{\pm,\rho} \equiv r v_j^\rho \) is the change in the number of \( X_j \) molecules due to the reaction \( r, \rho \). The chemical work done by the particle reservoirs in such a reaction step is

\[
W^{\tau,\rho}_\text{chem} \equiv W^{\tau,\rho}_\text{chem} \equiv -\sum_\alpha \mu_\alpha \Delta n_\alpha^{\tau,\rho} = \pm \sum_\alpha \mu_\alpha v_\alpha^\rho. \tag{18}
\]

where \( \Delta n_\alpha^{\tau,\rho} \equiv r v_\alpha^\rho \) is the number of \( A_\alpha \) molecules transformed in the reaction \( \pm, \rho \). Due to energy conservation, the heat flowing from the system into the heat bath follows as

\[
Q^{\tau,\rho} \equiv W^{\tau,\rho}_\text{chem} - \Delta E^{\tau,\rho} = -\sum_\alpha \mu_\alpha \Delta n_\alpha^{\tau,\rho} - r \sum_j E_j v_j^\rho. \tag{19}
\]

Thus, we have identified the first law of thermodynamics for a single reaction event as

\[
W^{\tau,\rho}_\text{chem} = \Delta E^{\tau,\rho} + Q^{\tau,\rho}. \tag{20}
\]
For a finite time interval $[0, t]$ with given concentration protocols $c_\alpha(\tau)$, we sum (17), (18) and (19) over all occurring reactions $\rho_l$ at times $\tau_l$, where we will denote forward reactions by $r_l = +1$ and backward reactions by $r_l = -1$. We then get the chemical work

\[ W_{\text{chem}} \equiv \sum_l \int_0^t d\tau (\sum_\alpha \mu_\alpha(c_\alpha(\tau))) \Delta n_{\alpha}^{r_l} = - \int_0^t d\tau \left( \sum_\alpha \mu_\alpha(c_\alpha(\tau)) \hat{n}_\alpha \right), \tag{21} \]

the change in internal energy

\[ \Delta E = \int_0^t d\tau \left( \sum_j E_j \hat{n}_j \right) \tag{22} \]

and the dissipated heat

\[ Q = -\Delta E - \sum_l \sum_\alpha \mu_\alpha(c_\alpha(\tau)) \Delta n_{\alpha}^{r_l} \]

\[ = - \int_0^t d\tau \left( \sum_j E_j \hat{n}_j + \sum_\alpha \mu_\alpha(c_\alpha(\tau)) \hat{n}_\alpha \right) \tag{23} \]

with

\[ \hat{n}_\alpha \equiv \sum_l \delta(\tau - \tau_l) r_l u_{\alpha}^{r_l} \tag{24} \]

and

\[ \hat{n}_j \equiv \sum_l \delta(\tau - \tau_l) r_l v_{j}^{r_l}. \tag{25} \]

Since only energies $E_j$ and chemical potentials $\mu_\alpha$ enter the crucial quantities $\Delta E$, $Q$ and $W$, this formulation of the first law is also valid in nonequilibrium.

The present identification of work, internal energy and heat depends crucially on the fact that we control the concentrations $c_\alpha$ externally. This means in particular that the chemical work has to be spent for “refilling” the chemiostats after each reaction step. A somewhat different identification applies if one considers relaxation (without further external interference) from an initially prepared nonequilibrium state with concentrations $c_{\alpha}^{\text{eq}}$ for $\tau \to \infty$. In this case, the system should comprise the $X_j$ and the $A_\alpha$ molecules. Then we cannot distinguish between the two types of species $X_j$ and $A_\alpha$ anymore and we have to label all reacting species with $\tilde{X}_j$, ($\tilde{j} = 1, \ldots, N_\alpha + N_j$) leaving no chemiostat species $\tilde{A}_\alpha$. The change in the internal energy of the system for a single reaction step then is

\[ \Delta \tilde{E} \equiv \sum_{\tilde{j}} \tilde{v}_{\tilde{j}}^{p} \tilde{E}_{\tilde{j}} \equiv \sum_{\tilde{j}} \tilde{v}_{\tilde{j}}^{p} E_{\tilde{j}} + \sum_\alpha u_{\alpha}^{p} E_\alpha \tag{26} \]

C. Relation between statistical and kinetic approach

In equilibrium, the chemical potentials $\mu_j$ must obey

\[ \sum_j v_{j}^{p} \mu_j = - \sum_\alpha u_{\alpha}^{p} \mu_\alpha = - \sum_\alpha u_{\alpha}^{p} [E_\alpha + T \ln (c^{eq}_\alpha \omega_j)] \tag{27} \]

since there is no chemical potential difference between connected states in equilibrium. We now compare the equilibrium distribution (11) to the grandcanonical equilibrium distribution of a dilute solution, which can always be written as (see App. A for details)

\[ p^{eq}(n, c^{eq}) = N \prod_j \frac{(\Omega/\omega_j)^{\nu_j}}{n_j!} e^{-\beta (E_j n_j - \mu_j n_j)} \tag{28} \]

with the energy $E_j$ of one $X_j$ molecule and appropriate normalization volumes $\omega_j$. Using the relations for the chemical potentials $\mu_j$ (24) and the system of linear equations for the $\epsilon_j$ (12), we see that thermodynamic consistency constrains the ratio of bare rates $k_\epsilon^{-}/k_\epsilon^{+}$ by

\[ \beta \sum_j v_{j}^{p} E_j = \ln \frac{k_\epsilon^{+}}{k_\epsilon^{-}} - \beta \sum_\alpha u_{\alpha}^{p} E_\alpha \tag{29} \]

where $\beta \equiv 1/T$ is the inverse temperature.

Two remarks are appropriate. First, in general the energies $E_j$ cannot be extracted from the kinetics, i.e. the chemical master equation, without knowledge of the detailed Hamiltonian since the bare rates $k_{\epsilon, \downarrow}^{\pm}$ depend on the normalizing volumes $\omega_\alpha$ and $\omega_j$ which are defined only through a microscopic Hamiltonian. Second, if one assumes the $k_{\epsilon, \downarrow}^{\pm}$ and the $\omega_\alpha$, $\omega_j$ to be given without specifying an underlying microscopic Hamiltonian, one has to make sure that the energies $E_\alpha$ are chosen such that eqs. (29) have a solution for the $E_j$. From another viewpoint, given energies $E_\alpha$ and $E_j$ constrain the possible bare rates for a given thermodynamically consistent reaction network. An illustration of this fact will be given in Sect. IV below.

IV. ENTROPY

We distinguish an entropy change of the system proper from a change in the entropy of the medium (heat bath). The total entropy production is the sum of both entropy changes.

A. Medium entropy change

The dissipated heat $Q$ from the first law corresponds to a change in entropy of the heat bath

\[ \Delta s_m = \frac{Q}{T} = - \frac{1}{T} \int_0^t d\tau \left( \sum_j E_j \hat{n}_j + \sum_\alpha \mu_\alpha \hat{n}_\alpha \right) \tag{30} \]
along a single trajectory. The change in medium entropy due to one occurring reaction \( \rho \) in direction \( r \) can be expressed through rate constants using the definitions of energies \( 29 \) and chemostat chemical potentials \( 15 \) as

\[
\Delta s_{m}^{r,\rho} = \frac{1}{T} Q^{r,\rho} = -r \beta \left( \sum_{j} E_{j} v_{j}^{\rho} + \sum_{\alpha} \mu_{\alpha} u_{\alpha}^{\rho} \right)
\]

\[
= r \ln \frac{k_+^{\rho}}{k_-^{\rho}} - r \sum_{\alpha} u_{\alpha}^{\rho} \ln (c_{\alpha} \omega_{\alpha})
\]

\[
= \ln \frac{w^{\rho}_{+}(\mathbf{n}, \mathbf{c})}{w^{\rho}_{-}(\mathbf{n} + r \mathbf{v}^{\rho}, \mathbf{c})} - r \sum_{\alpha} \ln \left( \frac{\Omega / \omega_{\alpha}}{(n_{\alpha} + r v_{\alpha})} \right)
\]

(31)

In the third line, we have used the transition probabilities \( 5 \) and \( 6 \). Summing \( 31 \) over all occurring reactions \( \rho_{l} \) in direction \( r_{l} \) at times \( \tau_{l} \) yields the medium entropy change

\[
\Delta s_{m} = \sum_{l} r_{l} \ln \frac{k_+^{\rho_{l}}}{k_-^{\rho_{l}}} - \sum_{l} r_{l} \sum_{\alpha} u_{\alpha}^{\rho_{l}} \ln (c_{\alpha} \omega_{\alpha})
\]

(32)

along a single trajectory.

**B. System entropy**

We will now define an entropy of the system. The total entropy production of system and heat bath then is the relevant quantity for the second law of thermodynamics. Again, we will be guided by the equilibrium case first. Equilibrium state functions can be obtained from \( 25 \) using the partition function

\[
Z = \prod_{\mathbf{n}} \frac{(\Omega / \omega_{j})^{n_{j}}}{n_{j}!} e^{-\beta (E_{j} n_{j} - \mu_{j} n_{j})}
\]

\[
= \prod_{\mathbf{n}} \exp \left( (\Omega / \omega_{j}) e^{-\beta (E_{j} - \mu_{j})} \right)
\]

(33)

and the grandcanonical potential

\[
J = -T \ln Z = -T \sum_{j} (\Omega / \omega_{j}) e^{-\beta (E_{j} - \mu_{j})}.
\]

(34)

Of particular importance is the equilibrium entropy of the system

\[
S_{\text{eq}} = -\partial_{T} J = - \sum_{\mathbf{n}} p^{\text{eq}}(\mathbf{n}) \left( \ln p^{\text{eq}}(\mathbf{n}) - \sum_{j} \ln \frac{(\Omega / \omega_{j})^{n_{j}}}{n_{j}!} \right)
\]

(35)

This expression differs by a term

\[
g_{\mathbf{n}} = \prod_{j} \frac{(\Omega / \omega_{j})^{n_{j}}}{n_{j}!}
\]

(36)

involving the degeneracy of the state \( \mathbf{n} \) from the usual Shannon entropy

\[
S_{\text{Sh}} = - \sum_{\mathbf{n}} p(\mathbf{n}) \ln p(\mathbf{n}) = \langle - \ln p(\mathbf{n}) \rangle
\]

(37)

of a Markov process. Relaxing the equilibrium constraint, we define the ensemble entropy in nonequilibrium as

\[
S(\tau) \equiv - \sum_{\mathbf{n}} p(n(\tau), \tau) \ln p(n(\tau), \tau) - \ln g_{\mathbf{n}}
\]

\[
= \langle - \ln p(n(\tau), \tau) + \ln g_{\mathbf{n}} \rangle.
\]

(38)

We will now generalize this ensemble expression to a stochastic entropy valid along a single trajectory for both equilibrium and nonequilibrium situations following \( 7 \). We define the stochastic system entropy

\[
s(\tau) \equiv - \ln p(n(\tau), \tau) + s^{0}(n(\tau)),
\]

(39)

where \( p(n(\tau), \tau) \) is the solution of the master equation \( 7 \) taken along the stochastic trajectory \( n(\tau) \) and

\[
s^{0}(n(\tau)) \equiv \ln g_{n(\tau)} = \sum_{j} \ln \left( \frac{\Omega / \omega_{j}}{n_{j}(\tau)} \right)
\]

(40)

is the internal entropy of the state \( \mathbf{n} \) due to the degeneracy. The ensemble entropy \( 38 \) then is the average of the stochastic entropy \( 39 \).

**C. Total entropy production**

The total entropy production along a stochastic trajectory

\[
\Delta s_{\text{tot}} = \Delta s + \Delta s_{m}
\]

\[
= \ln \frac{p(\mathbf{n}(0), 0)}{p(\mathbf{n}(t), t)} + \sum_{j} \ln \frac{(\Omega / \omega_{j})^{n_{j}(t)} n_{j}(0)!}{(\Omega / \omega_{j})^{n_{j}(0)} n_{j}(t)!}
\]

\[
+ \sum_{l} r_{l} \ln \frac{k_+^{\rho_{l}}}{k_-^{\rho_{l}}} - \sum_{l} r_{l} \sum_{\alpha} u_{\alpha}^{\rho_{l}} \ln (c_{\alpha} \omega_{\alpha})
\]

(41)

then is the sum of the changes of system entropy \( 29 \) and medium entropy \( 32 \), where \( \Delta s \equiv s(t) - s(0) \). It will be shown in Sect. \( \Box A \) below that, in fact, \( \Delta s_{\text{tot}} \) is independent of the normalizing volumes \( \omega_{\alpha} \) and \( \omega_{j} \). Hence, this quantity does not require explicitly a microscopic Hamiltonian but is rather determined by the full rates \( w_{+, -}(\mathbf{n}, \mathbf{c}) \) entering the master equation. As an aside, we note that after averaging the stochastic entropy production \( \Delta s_{\text{tot}} \), the ensemble entropy production from Ref. \( 4 \) is recovered.

**D. Stochastic Gibbs relation**

We can now relate the identified first law \( 20 \) to the usual equilibrium Gibbs relation. Inserting the definition
of the medium entropy [31] into [20], we find
\[ \Delta E = -T \Delta s_m - \sum_\alpha \mu_\alpha \Delta n_\alpha \] (42)

In equilibrium and for quasistatic transitions, we have \( \Delta s_{\text{tot}} = 0 \) and therefore \( \Delta s = -\Delta s_m \) which implies
\[ \Delta E = T \Delta s + \sum_j \mu_j \Delta n_j. \] (43)

Specialized to equilibrium, our approach thus yields along a single stochastic trajectory the usual thermodynamic relations valid for ensemble averages in equilibrium.

V. FLUCTUATION THEOREMS

Fluctuation theorems provide exact results for driven systems beyond linear response. So far, they have been studied extensively for Langevin dynamics and for master equations in general. Previous applications to chemical reaction networks [27, 41] have neither made the connection to an energy balance nor considered the entropy on the stochastic level with occasional exceptions [31, 49, 50, 54]. The purpose of this section is to develop this concept in full generality.

A. Integral fluctuation theorem

The weight \( p[\mathbf{n}(\tau), c(\tau)] \) for a particular trajectory \( \mathbf{n}(\tau) \) with given initial state \( \mathbf{n}_0 \) under the concentration protocol \( c(\tau) \) is given by
\[ p[\mathbf{n}(\tau), c(\tau)] = \frac{N_l}{\prod_{l=1}^{N_l}} \exp \left[ -\sum_{r, \rho} \int_{\tau_{l-1}}^{\tau_l} dr w^{\rho}_{\mathbf{n}_l \mathbf{n}_{l-1}}(\mathbf{n}_{l-1}, c(\tau)) \right] \times \prod_{l=1}^{N_l} w^{\rho}_{\mathbf{n}_l \mathbf{n}_{l-1}}(\mathbf{n}_{l-1}, c(\tau)), \] (44)

with \( \tau_l \) \((l = 1, \ldots, N_l)\) being the jump times, where a reaction \( \rho_l \) in direction \( r_l = +, - \) from state \( \mathbf{n}_{l-1} \) to state \( \mathbf{n}_l \equiv \mathbf{n}_{l-1} \pm \mathbf{v}^{\rho_l} \) occurs. For ease of notation, we have introduced the initial time \( \tau_0 = 0 \), the final state \( \mathbf{n}_{N_l+1} \equiv \mathbf{n}_N \) and the final time \( \tau_{N_{l+1}} \equiv t \). We then define
\[ R = \ln \frac{p_0(\mathbf{n}_0) \cdot p[\mathbf{n}(\tau), c(\tau)]}{p(\mathbf{n}, t) \cdot p[\mathbf{n}(\tau), c(\tau)]}, \] (46)
as the logarithm of the ratio of trajectory weights for a given trajectory \( \mathbf{n}(\tau) \) with initial state \( \mathbf{n}_0 \equiv \mathbf{n}(0) \) and final state \( \mathbf{n}_f \equiv \mathbf{n}(t) \) and the corresponding trajectory \( \tilde{\mathbf{n}}(\tau) \equiv \mathbf{n}(t - \tau) \) under the time reversed protocol \( \tilde{c}(\tau) \equiv c(t - \tau) \). Here, \( p_0(\mathbf{n}_0) \) is the initial distribution and \( p(\mathbf{n}, t) \) is the solution of the master equation at time \( t \). The integral fluctuation theorem
\[ \langle e^{-R} \rangle = 1 \] (47)
can then be proven by the following lines of identity [22, 27, 28, 31] using the normalization condition for the trajectory weight \( [41] \) and the final distribution \( p(\mathbf{n}, t) \)
\[ 1 = \sum_{\tilde{n}(\tau)} p(\mathbf{n}, t)p[\tilde{\mathbf{n}}(\tau), \tilde{c}(\tau)] = \sum_{\tilde{n}(\tau)} e^{-R[\tilde{n}(\tau)]} p_0(\mathbf{n}_0)p[\tilde{\mathbf{n}}(\tau), \tilde{c}(\tau)] = \sum_{\mathbf{n}(\tau)} e^{-R[n(\tau)]} p_0(\mathbf{n}_0)p[\mathbf{n}(\tau), c(\tau)] = \langle e^{-R[n(\tau)]} \rangle. \] (48)

Our thermodynamic approach allows to show that the abstract quantity \( R \) is exactly the total entropy production \( \Delta s_{\text{tot}} \). Inserting the trajectory weights \( [41] \) into \( [46] \) yields
\[ R = \ln \frac{p_0(\mathbf{n}_0)}{p(\mathbf{n}, t)} + \sum_l \ln \frac{\mathbf{n}_l}{\mathbf{n}_{l+1} \mathbf{n}_{l-1}^{-1} (\Omega/\omega)^{n_l^{-} - n_l^{+}}} \] (49)
\[ -\sum_l \ln \frac{n_{l+1}^{-1}}{n_l} \mathbf{n}^{+} - n_l^{-}) \] + \sum_l \ln \frac{w^{\rho}_{l-1}}{w^{\rho}_{l+1}}(\mathbf{n}_{l-1}, \mathbf{c}_l) \]
\[ \Delta s + \Delta s_m = \Delta s_{\text{tot}}. \] (50)

Here, we have used the shorthand notations
\[ \mathbf{n}' = \prod_j n_j! \] (51)
and
\[ (\Omega/\omega)^n = \prod_j (\Omega/\omega_j)^{n_j}. \] (52)

The key point in this analysis is the cancellation of the degeneracy terms from the change in system entropy \( \Delta s \) with those from the entropy change of the medium \( \Delta s_m \) in eq. \( [50] \). Thus, the total entropy production \( \Delta s_{\text{tot}} = R \) depends only on initial and final distribution \( p_0(\mathbf{n}_0), p(\mathbf{n}, t) \) and transition probabilities \( w^{\rho}_{l+1}(\mathbf{n}, \mathbf{c}) \), see eq. \( [49] \), which can, in principle, be extracted from experiments. Explicit knowledge of the normalizing volumes \( \omega_\rho \) and \( \omega_j \) defined only via the microscopic Hamiltonian is not required.
The fluctuation theorem
\[ \langle e^{-\Delta s_{\text{tot}}} \rangle = 1 \] (53)
then constrains the probability distribution of the total entropy production \( \Delta s_{\text{tot}} \) from which the second law
\[ \langle \Delta s_{\text{tot}} \rangle \geq 0 \] (54)
follows directly via the Jensen inequality \( \langle e^x \rangle \geq e^{\langle x \rangle} \). The relation (53) holds for any initial state, any time-dependent protocol \( c_\alpha(\tau) \) and any length \( t \) of trajectories. Similar relations have been derived within a Hamiltonian dynamics in Ref. 22.

B. Nonequilibrium steady states and detailed fluctuation theorem

Likewise, for nonequilibrium steady states with constant \( c_\alpha \), the detailed fluctuation theorem
\[ p(-\Delta s_{\text{tot}}) = e^{-\Delta s_{\text{tot}}} p(\Delta s_{\text{tot}}) \] (55)
can be shown exactly in the same fashion\(^{31}\). Here, \( p(\Delta s_{\text{tot}}) \) is the probability density function for the total entropy production. For the validity of this relation in the steady state for finite length \( t \) of trajectories, the inclusion of the stochastic entropy of the system \( \Delta s \) is crucial. In the long run, \( \Delta s_{\text{tot}} \) scales as \( t \) whereas \( \Delta s \) remains bounded.

C. Generalized Jarzynski relation

The Jarzynski relation\(^{20,21}\)
\[ \langle e^{-\beta W} \rangle = e^{-\beta \Delta F} \] (56)
expresses the free energy difference \( \Delta F \) between two equilibrium states with a non-standard average of the work \( W[x(\tau)] \) spent in this transition, which is a functional of the system trajectory \( x(\tau) \). In the form (56), it is valid only if the work is defined as
\[ W = \sum_k \int_0^t \frac{dE}{d\lambda_k} \lambda_k \] (57)
where the time-dependent protocols \( \lambda_k(\tau) \) (equivalent to the \( c_\alpha(\tau) \) in our context) determine the transition rates via the time-dependent potential \( E(\lambda_k(\tau), x) \). This definition of work is usually appropriate in a canonical ensemble. We now derive a generalized version of the Jarzynski relation for our grandcanonical system. We consider transitions between equilibrium states and rewrite the quantity \( \Delta s_{\text{tot}} \) using (20) and (30) as
\[ \Delta s_{\text{tot}} = \Delta s + \beta Q = \Delta s + \beta W_{\text{chem}} - \beta \Delta E \]
where we have used the definition of the grandcanonical potential
\[ \Delta J = \Delta E - T \Delta s + \sum_j \mu_j n_j. \] (59)

We now show that this change in the grandcanonical potential does not depend on initial \( (n_0) \) and final \( (n_f) \) state of the trajectory but only on the initial and final values of \( c \). Using the grandcanonical equilibrium distribution (28) and the definition of system entropy (39) we get
\[ J = E - Ts - \sum_j \mu_j n_j \]
\[ = \sum_j (E_j - \mu_j)n_j + T \ln p^{eq}(n, c) - \ln g_n + \ln N(c) \]
\[ = \sum_j (E_j - \mu_j)n_j - \sum_j (E_j n_j - \mu_j n_j) + \ln N(c) \]
\[ = \ln N(c) \] (60)
and therefore \( \Delta J = \ln N(c_f) - \ln N(c_0) \). Using (58), the fluctuation theorem turns into the generalized Jarzynski relation
\[ \langle e^{-\beta [W_{\text{chem}} - \Delta (\sum j \mu_j n_j)]} \rangle = e^{-\beta \Delta J}. \] (61)

Two remarks on the generality of this relation are appropriate.
(i) At constant \( c_\alpha \) belonging to an equilibrium state, the exponent on the lhs of eq. (61) can be transformed to
\[ W_{\text{chem}} - \Delta \left( \sum_j \mu_j n_j \right) \]
\[ = - \int_0^t d\tau \left( \sum_{\alpha} \mu_\alpha (c_\alpha^{eq}) \hat{n}_\alpha \right) - \Delta \left( \sum_j \mu_j n_j \right) \]
\[ = \sum_j \frac{d}{d\tau} \left[ \sum_{\alpha} \mu_\alpha (c_\alpha^{eq}) \hat{n}_j \right] - \Delta \left( \sum_j \mu_j n_j \right) \]
\[ = - \int_0^t d\tau \left( \sum_j \mu_j (c_\alpha^{eq}) \hat{n}_j \right) = 0 \] (62)
using the definitions of chemical work \( W_{\text{chem}} \) and chemical potential \( \mu_j \). Thus, the result (61) stays valid even if we do not await relaxation to the final equilibrium state. Such final relaxation implicitly assumed in the derivation of (61) does not contribute to the exponent on the rhs.
(ii) If we could clamp the initial and final number of molecules \( n_0 \) and \( n_f \), we would get the original relation
\[ \langle e^{-\beta W} \rangle = e^{-\beta \Delta F}. \] (63)
D. Generalized Clausius inequality

As mentioned above, there are two types of nonequilibrium in chemical reaction networks: (i) a genuine NESS and (ii) any type of time-dependent \(c_0(\tau)\). It is useful to split

\[
\Delta s_{\text{tot}} = \ln \frac{p_0(n_0)}{p(n_t, t)} + \sum_l \ln \frac{w^{p_l} (n_l^+, c_l)}{w^{p_l} (n_l^-, c_l)} = Y + \beta Q_{\text{hk}}
\]

into a \((c_0\text{-dependent})\) part

\[
Y = \ln \frac{p_0(n_0)}{p(n_t, t)} + \sum_l \ln \frac{p^0(n_l^+, c_l)}{p^0(n_l^-, c_l)}
\]

which accounts for non-adiabatic transitions and the so-called housekeeping heat\(^{30,49,51}\)

\[
\beta Q_{\text{hk}} = \sum_l \ln \frac{w^{p_l} (n_l^-, c_l)p^0(n_l^-, c_l)}{w^{p_l} (n_l^+, c_l)p^0(n_l^+, c_l)}
\]

which is the permanently dissipated heat in a nonequilibrium steady state for constant \(c_0\). Obviously, \(Q_{\text{hk}} = 0\) if the detailed balance condition (SB) holds for the stationary state \(p^0(n, c(\tau))\) for any time \(\tau\). For systems in a steady state, \(Y\) vanishes. For transitions between steady states, i.e., starting and ending in a stationary state, the quantity \(Y\) can be transformed to

\[
Y = \int_0^t d\tau \sum_\alpha \frac{\partial \Phi}{\partial c_\alpha} \phi_\alpha,
\]

with \(\Phi \equiv -\ln p^*\). It has been shown\(^{30,49}\), that the quantity \(Y\) obeys the integral fluctuation theorem (Hatanaka-Sasa-relation)

\[
\langle e^{-Y} \rangle = 1.
\]

From \((68)\) we get a generalized Clausius inequality even for transitions between nonequilibrium steady states

\[
\langle Y \rangle \geq 0
\]

or

\[
\beta \langle Q_{\text{ex}} \rangle \geq -\langle \Delta s \rangle
\]

with the excess heat

\[
\beta Q_{\text{ex}} \equiv \Delta s_m - \beta Q_{\text{hk}} = \sum_{l=1}^{k-1} \ln \frac{p^0(n_l^-, c_l)g_{n_l^-}}{p^0(n_l^+, c_l)g_{n_l^+}} = Y - \Delta s.
\]

The relation \((70)\) is a generalization of the classical thermodynamic Clausius inequality \(Q \geq -\Delta S\) to transitions between nonequilibrium steady states fitting in the phenomenological framework of steady state thermodynamics\(^{49,51}\). The fluctuation theorem

\[
\langle e^{-\beta Q_{\text{hk}}} \rangle = 1
\]

for the housekeeping heat has recently been proven for stochastic processes ruled by a Fokker-Planck equation\(^{56}\).

It can be shown that this relation holds also for chemical master equations\(^{52}\).

VI. AN EXAMPLE

We illustrate our general results by considering the simple model reaction network (see Fig. 2)

\[
A + X_1 \overset{k_+}{\rightarrow} X_2 \quad X_2 \overset{k_+}{\rightarrow} X_3 \quad X_3 \overset{k_+}{\rightarrow} X_1 + B
\]

where \(A\) and \(B\) are chemostat species (corresponding to \(A_1\) and \(A_2\) in our previous notation). For simplicity, we assume that all normalizing volumes \(\omega_j\ (j = 1, 2, 3)\) in principle defined through a microscopic Hamiltonian, see App. A, are equal. These normalizing volumes connect the bare rates \(k_{\pm j}\) to the transition rates \(\omega_j^0(n, c)\) through \((5)\) and \((6)\). The chemostat species have chemical potentials\(^{14}\)

\[
\mu_A = E_A + T \ln (c_A \omega_A)
\]

and

\[
\mu_B = E_B + T \ln (c_B \omega_B)
\]

where \(E_A\) and \(E_B\) are single particle energies of \(A\) and \(B\) species, respectively, and \(\omega_A, \omega_B\) are appropriate normalizing volumes. The stoichiometric matrix \(V = (v_{ij})\)

\[
V = \begin{pmatrix}
-1 & 1 & 0 \\
0 & -1 & 1 \\
1 & 0 & -1
\end{pmatrix}
\]

(\(\rho = 1\))

(\(\rho = 2\))

(\(\rho = 3\))

(76)

gives the number of transformed \(X_j\) molecules in one forward reaction of type \(\rho\). Obviously, it is singular with \(\text{rank } V = 2\), which corresponds to case II from Sect. II B. We therefore have the constraint

\[
n_1 + n_2 + n_3 = N
\]

(77)

on the accessible states of the phase space. Thus, the total number \(N\) of \(X\)-molecules is a conserved quantity.
A. Equilibrium distribution

In order to determine the potential equilibrium distribution, we try to solve eqs. \[ (12) \]
\[
\begin{pmatrix}
-1 & 1 & 0 \\
0 & -1 & 1 \\
1 & 0 & -1
\end{pmatrix}
\begin{pmatrix}
\epsilon_1 \\
\epsilon_2 \\
\epsilon_3
\end{pmatrix}
= \begin{pmatrix}
-\ln(c_A\omega_A) + \ln(k_-/k_+) \\
\ln(k_-/k_+) \\
\ln(c_B\omega_B) + \ln(k_-/k_+)
\end{pmatrix}
\]
which have solutions only if
\[
c_A/c_B = (\omega_B/\omega_A)(k_-/k_+)^3 \equiv c_A^q/c_B^q.
\] (79)

This is the expected condition for equilibrium since for any network cycle obeying detailed balance the product of forward and backward rates must be equal. We then get the solution
\[
\begin{pmatrix}
\epsilon_1 \\
\epsilon_2 \\
\epsilon_3
\end{pmatrix}
= \begin{pmatrix}
\ln(c_A\omega_A) - \ln(k_-/k_+) \\
0 \\
\ln(k_-/k_+)
\end{pmatrix} + \gamma \cdot \begin{pmatrix}
1 \\
1 \\
1
\end{pmatrix},
\] (80)
with arbitrary \(\gamma\). The equilibrium distribution is the projection of the Poisson distribution \(10\) to the accessible state space, i.e. the states with total number \(N\) of \(X\)-molecules. This yields
\[
p^{eq}(n, c) \propto \prod_j \frac{(\Omega/\omega_j)^n_j}{n_j!} e^{-c_j n_j} \delta_{N,n_1+n_2+n_3} \times \frac{\delta_{N,n_1+n_2+n_3}}{n_1! n_2! n_3!} \left( \frac{k_-}{k_+ c_A \omega_A} \right)^{n_1} \left( \frac{k_+}{k_-} \right)^{n_3}.
\] (81)

B. Energy

In principle, the energies \(E_j\) of one molecule \(X_j\) are defined as the thermodynamic average of the microscopic Hamiltonian, see App. \[A\]. Without specifying such a Hamiltonian, for given bare rates \(k_{+,-}\), the energies \(E_j\) of a single solute molecule \(X_j\) must obey \[29\]
\[
\begin{pmatrix}
-1 & 1 & 0 \\
0 & -1 & 1 \\
1 & 0 & -1
\end{pmatrix}
\begin{pmatrix}
E_1 \\
E_2 \\
E_3
\end{pmatrix}
= \begin{pmatrix}
\ln(k_-/k_+) \\
\ln(k_-/k_+) \\
\ln(k_-/k_+)
\end{pmatrix} - \begin{pmatrix}
-E_A \\
0 \\
E_B
\end{pmatrix}
\]
which has a solution for the \(E_j\) only if
\[
E_B - E_A = 3 \ln \frac{k_-}{k_+}.
\] (83)

This is the condition for the reaction network to be thermodynamically consistent as discussed in Sect. \[IIIC\]. To simplify matters, we have set here and in the following the inverse temperature \(\beta = 1\).

The difference in energies \(E_A\) and \(E_B\), combined with entropic forces, is the driving force of a potential nonequilibrium steady state. If the energies were equal, there was no reason for the reaction to run on average in one direction for equilibrated concentrations \(c_A\omega_A = c_B\omega_B\) and hence the bare rates \(k_+\) and \(k_-\) were equal. The ratio of bare rates \(k_-/k_+\) thus depends on the reservoir energies through \[33\]. We then get for the relationship between energies \(E_j\) and bare rates \(k_{+,-}\)
\[
\begin{pmatrix}
E_1 \\
E_2 \\
E_3
\end{pmatrix}
= \begin{pmatrix}
E_0 - E_A \\
E_0 + \ln(k_-/k_+) \\
E_0 + 2\ln(k_-/k_+)
\end{pmatrix}
\]
with arbitrary \(E_0\).

Having obtained single particle energies in an equilibrium situation, the reaction system can now be driven out of equilibrium by changing the concentration of \(A\) or \(B\) molecules. Non-adiabatic transitions between equilibrium distributions are achieved by driving \(c_A(\tau) = (\omega_B/\omega_A)(k_-/k_+)^3c_B(\tau)\). Non-equilibrium steady states and transitions between them are obtained for any protocol with \(c_A(\tau) \neq (\omega_B/\omega_A)(k_-/k_+)^3c_B(\tau)\).

C. First law

Whenever a single reaction takes place, the chemiostats associated with \(A\) and \(B\) molecules apply work to the system consisting of the \(X_j\)-molecules. Furthermore, the system of the \(X_j\)-molecules dissipates heat into the surrounding aqueous solution acting as a heat bath. The chemical work \(W_{chem}\) done by the chemiostats due to a single reaction is generally given by \[18\] as
\[
W_{chem}^{\tau,\rho} = -\sum_\alpha \mu_\alpha \Delta n_\alpha^{\tau,\rho}.
\] (85)

Due to energy conservation, the dissipated heat for one reaction step is given by \[19\] as
\[
Q^{\tau,\rho} = W_{chem}^{\tau,\rho} - \sum_j E_j \Delta n_j^{\tau,\rho}.
\] (86)

Having defined work, dissipated heat and internal energy, we can formulate the first law in the form \[20\]
\[
W_{chem}^{\tau,\rho} = \Delta E^{\tau,\rho} + Q^{\tau,\rho}.
\] (87)

Chemical work, dissipated heat and change of internal energy for one single forward reaction step of each type \((\rho = 1, 2, 3)\) are shown in Tab. \[\] The expressions for the heat agree with the general result \[31\]. The dissipated heat for a given sequence of reactions thus depends on the concentrations \(c_A\) and \(c_B\). In equilibrium, the dissipated heat is zero for a complete cycle and therefore is zero on average. However, for a single reaction step the heat is not necessarily zero.

D. Entropy production

For an illustration of the entropy change, we calculate the total entropy production \[31\] due to one occurring
from state \( n^- \) to state \( n^+ \) at time \( \tau \). The total entropy production \( \Delta s_{\text{tot}} \) is the sum of the change in system entropy and the entropy change of the heat bath:

\[
\Delta s = -\ln p(n^+, \tau) + s^0(n^+) + \ln p(n^-, \tau) - s^0(n^-)
\]

\[
= \ln \frac{p(n^-, \tau) n_3}{p(n^+, \tau)(n_1 + 1)}
\]

and the entropy change of the heat bath:

\[
\Delta s_m = Q = \ln \left( \frac{k_+}{k_- c B \omega_B} \right).
\]

The total entropy production then is

\[
\Delta s_{\text{tot}} = \Delta s + \Delta s_m
\]

\[
= \ln \frac{p(n^-, \tau)}{p(n^+, \tau)} + \ln \frac{n_3 k_+}{(n_1 + 1) k_- c B \omega_B}
\]

\[
= \ln \frac{p(n^-, \tau) \omega^e_x(n^-, \mathbf{c} (\tau))}{p(n^+, \tau) \omega^e_x(n^+, \mathbf{c} (\tau))} = R.
\]

This illustrates the cancellation of the degeneracy term and the equivalence of the abstract quantity \( R \) and the thermodynamically motivated entropy production \( \Delta s_{\text{tot}} \). The total entropy production thus could be extracted from experiments if the state probabilities and the transition probabilities of the stationary state could be measured.

\textbf{VII. CONCLUSION AND PERSPECTIVES}

In summary, for arbitrary chemical networks in a dilute solution described by a chemical master equation, we have developed a consistent thermodynamic description along a single stochastic trajectory by identifying work, internal energy and dissipated heat for each reaction step. A unique identification of internal energy and dissipated heat requires a microscopic Hamiltonian for the interaction between the reacting species and the solvent and cannot be extracted from the stochastic dynamics alone. However, we have shown that work and total entropy production do not depend on the microscopic details and can be extracted from the dynamics once the transition rates in the chemical master equation are specified or measured in an experiment. Entropy production involves both the change of the entropy of the medium given by the dissipated heat and genuine entropy of the network which requires a proper consideration of a degeneracy factor. The total entropy production fulfills an integral fluctuation theorem for arbitrary driving and a detailed fluctuation theorem in the steady state.

This systematic approach relies on three crucial concepts. First, an energetic interpretation of stochastic master equation dynamics requires the comparison with an appropriate equilibrium state whose microscopic Hamiltonian for the interaction with the solvent must be known. By choosing the equilibrium concentrations of reservoir particles and thus adjusting the net flux to zero, a stationary distribution obeying detailed balance can be obtained for any thermodynamically consistent reaction network. Second, the persistence of mass action law kinetics even in non-equilibrium leads to the concept that energy differences are related to the ratio of bare rates in the same way as in equilibrium. Crucial for the persistence of this assumption is the time scale separation of diffusion and reaction time constants. For higher concentrations or long range interactions, a coupled system of reaction-diffusion equations would be necessary. The general concepts of stochastic thermodynamics would still apply; however, the expressions for energy, dissipated heat, work and entropy would get more complex. Third, we have decided to define the system as the collection of those species whose numbers we can follow in principle. The internal energy then is associated with these molecules only. This third assumption could be somewhat relaxed since one could always include one of the chemistat species to the system species. The first two assumptions, however, are crucial if one wants to keep the energetic interpretation. Of course it is still possible to derive integral and detailed fluctuation theorems for a stochastic entropy production in any network obeying master equation dynamics. The interpretation of the abstract quantity \( \Delta s_m \) as an exchanged heat, however, requires the first law energy balance and the correct identification of the degeneracy factor.

Distributions of work or entropy production may be used to reconstruct thermodynamic equilibrium quantities or to characterize the network, similar to the reconstruction of free energy landscapes for unzipping or unfolding transitions using the Jarzynski relation. A first step in this direction is the use of fluctuation theorems to determine chemical driving forces in reaction cycles. Of particular interest would also be the derivation of the distributions of work and total entropy production for frequently used models of gene regulation networks or signal processing networks, especially since it is known from a simple athermal model system that these distributions can show a quite rich structure.
perimental work on fluctuation theorems for Langevin-systems, we are not aware of any attempts to probe fluctuation theorems for chemical reaction networks experimentally. Such experiments could contribute substantially to the ongoing effort to achieve a better understanding of nonequilibrium systems.

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**APPENDIX A: NORMALIZING VOLUMES AND THERMODYNAMICS OF A DILUTE SOLUTION**

For a precise definition of the normalizing volumes \( \omega_{\alpha}, \omega_j \) and the energies \( E_\alpha, E_j \), we will now briefly review the standard thermodynamics of a dilute solution as found in textbooks, see e. g. [2]. For ease of notation, we treat the case of a dilute solution of \( N' \) equivalent solute molecules in a solvent with \( N \) molecules. This calculation can easily be generalized to the case of more than one species and applies both to the Hamiltonian and to the calculation can easily be generalized to the case of more than one species and applies both to the Hamiltonian and to the ongoing effort to achieve a better understanding of nonequilibrium systems.

We assume that the number of solvent molecules \( N \) is fixed and therefore use a semi-grandcanonical ensemble. In the dilute limit \( N' \ll N \), the Hamiltonian can then be split into the contributions

\[
H^N,N'(\{r\}, \{r'\}) = H^N_1(\{r\}) + H^{N'}_1(\{r'\}) + H^{N,N'}_1(\{r\}, \{r'\}) \tag{A1}
\]

with

\[
H^{N'}_1(\{r'\}) = \sum_{i=1}^{N'} H_1(r'_i) \tag{A2}
\]

and

\[
H^{N,N'}_1(\{r\}, \{r'\}) = \sum_{i=1}^{N'} H_1(r'_i, \{r\}) \tag{A3}
\]

where \( r'_i \) denotes the position of the \( i \)th solute particle with \( i = 1, \ldots, N' \) and \( \{r\} \) are the coordinates of the solvent molecules. One particle energies \( E_i \) (\( i = \alpha, j \)) are defined through

\[
E_i = \langle H_1(r'_i) + H_1(r'_i, \{r\}) \rangle_{N, N'} \tag{A4}
\]

Here, \( \langle \cdot \rangle \) denotes the canonical average with the appropriate canonical Boltzmann-weight.

Having defined one particle energies \( E_\alpha \) from a microscopic point of view through \( \langle \cdot \rangle \), one can transform the chemical potential \( \omega_\alpha \) into the form \( \omega_\alpha \) by choosing an appropriate normalizing volume

\[
\omega_\alpha \equiv e^{\beta(E^0_\alpha - E_\alpha)}/c_0. \tag{A5}
\]

where \( \beta \equiv 1/T \) is the inverse temperature.

We now recall how to obtain the grandcanonical equilibrium distribution \( p^eq(N') \). Integrating out the spatial degrees of freedom, we get

\[
p^eq_N(N') = \frac{Z_{N}^{N'} e^{-\beta \mu N'}}{\sum_{N'} Z_{N}^{N'} e^{-\beta \mu N'}} \tag{A6}
\]

where \( Z_{can}^{N,N'} \) is the canonical partition function which can be calculated as

\[
Z_{can}^{N,N'} = F(1) \frac{\Omega N}{\lambda^3} \Psi(T, \Omega/N) \tag{A7}
\]

where \( \Psi(T, \Omega/N) \) and \( F(1) \) depend on the particular Hamiltonian and \( \Omega \) is the thermal de Broglie wavelength of a solute particle. The solute molecules thus behave like an ideal gas in the effective (constant) potential \( \Psi(T, \Omega/N)/\beta \). Inserting (A7) into (A6) yields

\[
p^eq_N(N') \propto \frac{1}{N!/\lambda^3 e^{-\beta E}} \Psi(N') e^{\beta \mu N'} \tag{A8}
\]

Thus, Eq. (A8) has the form of eq. (28) with \( \omega \equiv \lambda^3 e^{-\beta E}/\Psi \). A simple calculation shows that eqs. (15) and (A6) are consistent with eq. (A3).

**APPENDIX B: ANALYSIS OF CASE II FROM SECT. III**

If there exists more than one solution of (12), the rank of the stochiometric matrix \( V \) is smaller than the number \( N_j \) of species \( X_j \). This is usually the case if there are less reactions \( \rho \) than species \( X_j \), i.e. \( N_\rho < N_j \). We then have \( N_\mu = N_j - rank V \) (generically : \( N_\mu = N_j - N_\rho \)) constraints

\[
\sum_j K^{\mu}_{j} n_j = L^\mu \tag{B1}
\]

\( \mu = (1, \ldots, N_\mu) \) for the accessible states with \( L = (L^1, \ldots, L^{N_\mu}) \) depending on the initial distribution. The matrix \( K \) has rows which span the nullspace of the stochiometric matrix \( V \)

\[
\sum_{j=1}^{N_j} (v^\mu_j) K^{\mu}_{j} = 0. \tag{B2}
\]

The general solution of (12) then is the sum of a special solution \( \epsilon^\mu_0 \) and a nullspace vector \( K x \)

\[
\epsilon_j = \epsilon^\mu_0 + \sum_{\mu=1}^{N_\mu} K^{\mu}_{j} x^{\mu} \tag{B3}
\]
for an arbitrary vector $\mathbf{x} \in \mathbb{R}^d$. The state space $\{\mathbf{n}\}$ is then separated into an infinite number of subspaces $\chi_L = \{\mathbf{n} | \mathbb{R}^d n_j = L^\mu \} \subset \{\mathbf{n}\}$. If all possible initial states lie in the same subspace $\chi_L$, the equilibrium distribution is the projection of the Poissonian (13) to the subspace $\chi_L$ with the $\epsilon_j$ given by any solution of (12). It does not matter which solution of (12) is chosen since

$$p^{eq}(\mathbf{n}, \mathbf{c}) = N^\prime \prod_j (\Omega/\omega_j)^{n_j} n_j! e^{-\epsilon_j n_j}$$

$$= N^\prime \prod_j (\Omega/\omega_j)^{n_j} n_j! e^{-(\epsilon_j + \sum_n K_{nj} x_n)n_j}$$

$$= N^\prime \prod_j (\Omega/\omega_j)^{n_j} n_j! e^{-\epsilon(n_j)L_j}$$

$$= N^\prime \prod_j (\Omega/\omega_j)^{n_j} n_j! e^{-\epsilon(n_j)}. \quad \text{(B4)}$$

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