Generalized Boltzmann Distribution for Systems Out of Equilibrium

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Abstract

The theory of statistical mechanics pioneered by Boltzmann has enabled predictive understanding of a wide range of physical systems that are at thermal equilibrium. Understanding how living systems function at the microscopic scale requires an equally powerful theory of far-from-equilibrium behavior. Such a theory has so far been lacking. Here, I generalize the Boltzmann distribution to enable calculation of microstate probabilities and fluxes for systems that are driven, time-dependent, and/or are not at steady state; at equilibrium, the equation reduces to the Boltzmann distribution. The derived equation is an isomorphic mapping between any network of microstates to an equivalent electrical circuit with resistors and voltage-dependent voltage sources. This mapping allows for the application of powerful theorems from circuit theory to nonequilibrium statistical mechanics, from which they take on new meaning, such as maximum efficiency and reciprocal relations arbitrarily far from equilibrium. These relations are obeyed by driven steady state systems in general, and therefore govern biological performance, response, and evolvability. The resistors can be systematically coarse-grained to simplify the analysis of complex systems, as well as to deduce microscopic thermodynamic information like free energy barriers from measurements of macroscopic observables like dissipation rate or motility. As concrete examples, the single-cycle microstate topology with one and two driving sources, which describe protein catalysis and activation, respectively, are solved analytically. In these cases, being driven far from equilibrium allows function to be optimized independently of stability.

I. INTRODUCTION

The phase space of a system with interacting degrees of freedom consists of all possible states of the system and grows exponentially with the number of degrees of freedom. If the system is in thermodynamic equilibrium, then it obeys detailed balance, which means that the ensemble-averaged forward rate of transitioning between any two states is exactly balanced by the reverse rate, yielding zero net current between all states in the phase space. The probability, \( P_i \), of finding the system in state \( i \) as opposed to state \( j \) is determined by the Boltzmann equation [1]:

\[
P_i e^{βG_i} = P_j e^{βG_j}, \tag{1}
\]

where \( G_i \) is the free energy of state \( i \) and \( β \) is the inverse of the Boltzmann constant times the temperature. Eq.1 is powerful because it shows how the relative probability of any state (a collective property over all the states) is determined by its free energy (a local property of the state) at equilibrium.

At sub-cellular length scales, biological systems are composed of macromolecules such as proteins, and each state of the system is defined by specifying the relative abundances of all macromolecules relevant to the system as well as their conformational (i.e. folding) and chemical modification (e.g. phosphorylation) status. The energy to transition between states is typically comparable to thermal noise, and in the absence of net energy flow between the system and its environment, the system would attain a state of thermal equilibrium. Yet most biological processes are not at equilibrium because energy is constantly being fed in to drive them, and as a result their microstate probabilities do not obey the Boltzmann distribution. Nevertheless, they do seem to occupy a small subset of function-
ally relevant states within their enormous phase space, and to transition between such states in a reproducible way. Such a system can still reach a steady state that does not obey detailed balance, resulting in net fluxes between microstates capable of performing work and dissipating heat. A wide variety of microscopic life processes use the hydrolysis of ATP into ADP (or GTP into GDP) as an energy currency; such processes are driven by the continual net recycling of spent ADP back to ATP.

A theory, akin to the Boltzmann distribution, is needed to relate nonequilibrium fluxes and probabilities to microscopic parameters such as energies and rate constants. These parameters can be changed via biochemical regulation or genomic mutation on the timescales of organisms and species, respectively. The lack of such a theory has limited our ability to answer fundamental questions about biology. Such questions include: why are living systems generically so efficient at performing complex tasks despite thermal stochasticity, for example when transporting cargo, pumping ions or synthesizing proteins [2, 3, 4, 5]? Is it necessary for life to operate far from equilibrium? How are biological systems simultaneously robust and complex? Are there simple effective variables that can be systematically tuned or is the irreducible set of variables generally very large? Are there invariant physical constraints on how systems must behave or evolve?

To date, the closest we’ve come to answering such questions are for systems near, or tethered to, equilibrium conditions. These include the relationship between the work necessary to transition a system from one equilibrium state to another and the free energy difference between the two states [6], as well as the principle that systems behave in a way that extremizes entropy or path entropy when driven close to equilibrium (such that the current has a linear response to the driving force) [7, 8, 9, 10]. Far from equilibrium, such variational principles are not sufficient for predicting system behavior in general [11]. The fluctuation theorems, which are valid far from equilibrium, are not predictive without knowing the nonequilibrium microstate probabilities [12, 13, 14]. A graph theory-based approach exists to compute these probabilities and fluxes at steady state [15, 16]. However, this method is a recipe of combinatorial graph calculations, in which the number of system-specific sub-graphs scales super-exponentially with the number of microstates [17]. These restrictions limit the ability of this approach to establish general properties, and is computationally impractical.

Here, I show that there is a generalization of the Boltzmann distribution, valid for all thermostatted systems, including those that are strongly driven as well as time-varying. The equation gives nonequilibrium probabilities and net fluxes as a function of equilibrium free energies and the rate constants of driven and un-driven transitions. Remarkably, this generalization is a mapping from the microstate network of a given nonequilibrium system to an electronic circuit whose nodes are the microstates and whose resistances and voltages correspond to the equilibrium and driven components of the system, respectively. The resistances in a network can be systematically coarse-grained into a few effective resistors, enabling exact coarse-graining of complex systems. New laws of nonequilibrium steady states, such as far-from-equilibrium reciprocal relations and upper bounds on efficiency, can be found which hold for any system driven arbitrarily far from equilibrium by an external energy source. The generalized Boltzmann distribution shows how desired states can be amplified orders of magnitude beyond their equilibrium probabilities and how the state probabilities and currents depend on temperature, driving rate, and the free energy landscape of the non-driven system. For systems described by a cyclic microstate topology, I explicitly show how they can convert local stability and control at equilibrium into global stability and control when driven far from equilibrium, thereby freeing systems to evolve functionality independent of stability optimization. Many of these properties are shown to depend on being driven far from equilibrium.
II. THE GENERALIZED BOLTZMANN DISTRIBUTION

Consider a system capable of existing in \( N \) microstates (Fig. 1a). Let the rate coefficients between the microstates be such that the system will reach thermodynamic equilibrium. Define two microstates \( m \) and \( n \) to be "neighbors" if there exists a pathway between them that does not traverse any other microstates. The system is then completely characterized by forward rate constants between neighboring states \( k_{mn} \) and free energies \( G_m \); \( k \)'s will be referred to as "equilibrium rate constants." Any nonequilibrium system can be constructed by adding driven rate constants \( \alpha_{mn} \) to the rate constants of an original equilibrium system. The net current, \( I_{mn} \), from state \( m \) to \( n \) is

\[
I_{mn} = P_m (k_{mn} + \alpha_{mn}) - P_n (k_{nm} + \alpha_{nm}),
\]

which is the sum of the forward and reverse rates. At equilibrium, all \( \alpha_{mn} \) are zero. In the case that some \( \alpha_{mn} \) are nonzero, the system may reach a nonequilibrium steady state with, in general, non-zero currents.

Define a "voltage" source between neighboring states as:

\[
\mathcal{E}_{mn} = \alpha_{mn} \frac{e^{\beta G_m} P_m - e^{\beta G_n} P_n}{k_{mn}},
\]

which is zero when the transition between \( m \) and \( n \) is not driven. Define the resistance between any two neighbor states \( m \) and \( n \):

\[
R_{mn} = \frac{e^{\beta G_m} k_{mn}}{k_{nm}} = R_{nm}.
\]

Figure 1: Microstates form circuits in phase space. The graph structure and transition rate constants between states determine the likely trajectories of the system in phase space (a). Due to the mapping between microstate circuits and electrical circuits, the microscopic parameters that determine the transition dynamics become equivalent to circuit elements such as voltage sources and resistors, which can be systematically coarse-grained to reveal the essential description of system behavior (b).

Note that the second equality in Eq. 3 follows because the equilibrium forward rate constant is equal to the equilibrium backward rate constant times the exponential of the free energy difference between the states; hence the resistance is directionally symmetric. This sym-
metry is crucial for the mapping to a resistive circuit, and holds even when the network is being driven such that the directional symmetry of the currents (i.e. detailed balance) is broken.

The generalized Boltzmann equation for any states $i$ and $j$, which are not necessarily neighbors, is obtained by summing all $R_{mn}I_{mn}$ on any path between $i$ and $j$, substituting Eqs. 2-3, and canceling terms:

$$P_i e^{\beta G_i} - P_j e^{\beta G_j} = \sum_{m=i}^{n=j} \mathcal{E}_{mn} - R_{mn} I_{mn}, \tag{4}$$

where the sum is over any path between states $i$ and $j$, with $m$ and $n$ denoting intermediate microstates on the path. Eq. 4 generalizes the Boltzmann distribution to driven and time-dependent systems. It is the principal result of this work, and the one from which all subsequent results are derived. The “voltage potential” at microstate $i$ is the ratio of the probability of state $i$ divided by its equilibrium probability: $V_i \equiv P_i e^{\beta G_i} = P_i Z / Z'$, where $P_i^*$ and $Z$ are the equilibrium probability of state $i$ and the partition function, respectively. As defined in Eq. 2, this means that the voltage source, $\mathcal{E}_{mn}$, is itself proportional to the voltage potential at $m$, and is an example of a voltage-dependent voltage source. It is this feedback between the voltage and the voltage source that qualitatively differentiates linear near-equilibrium behavior from nonlinear far-from-equilibrium behavior. Eq. 4 applied to any loop in phase space gives zero net change in voltage: $\sum_{\text{loop}} \mathcal{E}_{mn} - R_{mn} I_{mn} = 0$, i.e. Kirkhoff’s “loop rule.” At steady-state, Kirkhoff’s “junction rule” also applies: the sum of all net fluxes into each state is zero (in electrical circuits, this criterion is true even when not at steady state due to zero net charge in any discrete circuit element). At steady state, the number of equations necessary to solve is equal to the number of simple loops in the microstate network (e.g. using the method of mesh currents) plus the number of voltage sources (which are voltage-dependent). The number of equations to solve could therefore be much fewer than the number of microstates of the system. Furthermore, by the methods to combine resistors in parallel, in series, or via the star-mesh transform, the number of effective variables can be reduced without losing the explicit dependence on the elementary parameters (Fig.1b) [18].

In the special case that there are no driven rates, $I_{mn} = 0$ for all $m$ and $n$, then $I_{mn} = 0$ everywhere at steady state. Otherwise, the steady-state condition necessitates at least one closed loop with currents all flowing in the same direction, in violation of Eq. 4 applied to the closed loop. Therefore, if all driven rates are zero, Eq. 4 reduces to the Boltzmann distribution Eq. 1.

Zia and Schmittmann, drawing an analogy with electric circuits, postulated that the full characterization of nonequilibrium steady states required that the interstate currents be placed on equal footing with the probability distribution [17]. Eq. 4 shows that this analogy becomes an exact mapping when the voltage and resistance are defined as in Eqs. 2-3. In this mapping, equilibrium corresponds to the situation in which all microstates are equipotential. Due to the absence of net current at equilibrium, the free energies, being conjugate to the probabilities, are the only important microstate parameters. Out of equilibrium, the resistances, being conjugate to the currents, are just as important as free energies.

### III. Steady State Theorems

For any system driven by thermal stochasticity as well as biasing forces, Eq. 4 shows that the flow and distribution of probability in the system’s state space behaves in the same way as the current and voltage distribution in an electrical circuit; the nondriven and driven transitions correspond to “passive” resistors and “active” voltage sources. This mapping allows for the cross-application of general theorems from the field of circuits, many of which hold for arbitrarily complex circuits. Here, two such theorems, Tellegen’s Theorem and Lorentz’s Reciprocity Theorem, are used to derive new statiststical mechanical relations that hold for any system driven arbitrarily far from equilibrium by a single source.
1. Entropy Production and Maximum Efficiency

It is useful to describe the energy dissipation of a given system in terms of the circuit formalism presented here. From the fluctuation theorem \([12]\), the entropy production rate of the system is:

\[ \sigma = \sum_{ij} I_{ij} \ln \left( \frac{P_i}{P_j} \right), \]

with the logarithm of the ratio of forward and reverse rates termed the "affinity" \([16]\). This can be rewritten as:

\[ \sigma = \sum_{ij} I_{ij} \left( \ln \left( \frac{P_i}{P_j} \right) - \ln \left( \frac{P_j}{P_i} \right) \right) + \sum_{ij} I_{ij} \ln \left( 1 + \frac{\alpha_{ij}}{k_{ij}} \right). \]

At steady state, the currents obey Kirchoff's current law, and the first summation is zero \([19]\). The entropy production rate at steady state is thus:

\[ \sigma = \sum_{ij} I_{ij} \ln \left( 1 + \frac{\alpha_{ij}}{k_{ij}} \right), \]

which can be computed by measuring the currents of only the directly driven processes (for which \(\alpha_{ij} > 0\)). It is thus categorically simpler than obtaining the currents and probabilities of all states in order to calculate the entropy production before Tellegen's theorem is applied.

Consider a singly driven system, for which there is a single nonzero driven rate constant, denoted \(\alpha_d\). If we define all other transitions as the "load" of the microstate network, then the entropy production of the load is equal to the total entropy production minus the entropy production of the driven transition. Whether for engineered or evolved systems, the power source (directly driven transitions) are typically optimized separately from the load, allowing each to be independently improved and modularly combined. In the state space of nonequilibrium systems, the load comprises the set of states or transitions that are the ultimate goal of driving the system. In biology, the load could correspond to a particular cellular pattern, protein assembly, or directed movement, that is negligible at equilibrium. Running current through the load activates nonequilibrium processes (what Prigogine called "dissipative structures"), including those in living systems \([20]\). The entropy produced by the load at steady state is:

\[ \sigma_{\text{load}} = I_d \ln \left[ 1 + \left( \frac{R_{d,\text{Load}}}{R_d + R_{d,\text{Load}}} \right) \frac{\alpha_d}{k_d} \right], \]

where the load resistance, \(R_{d,\text{Load}}\), is the total resistance of the remainder of the circuit if \(R_d\) were replaced with a voltage source with zero resistance; in circuit theory the load resistance is called the Thevenin resistance \([21]\). \(\sigma T\) is the rate that heat is dissipated by the entire microstate circuit, whereas \(\sigma_{\text{load}} T\) is the rate that heat is dissipated by the load.

Near equilibrium, substituting \(I_d \approx (R_d + R_{d,\text{Load}})^{-1}(\alpha_d/k_d)\) into Eq. 6 and only keeping first order terms in \(a/k\) gives:

\[ \sigma_{\text{load}} = \frac{R_{d,\text{Load}}}{(R_d + R_{d,\text{Load}})^2} \left( \frac{\alpha_d}{k_d} \right)^2 \leq \frac{1}{4kR_d} \frac{\alpha_d}{k_d}. \]

The maximum dissipation rate of the load in this inequality is achieved when the load resistance is equal to the driven resistance. This condition is analogous to the maximum power transfer theorem in electrical circuits, in which the load resistance must be equal to the internal resistance of the source in order to dissipate the maximum amount of power in the load. For large driving forces, the response current \(I_{ij}\) will no longer be linear in \(a/k\), and the optimal load resistance will in general depend on the network.

Using Eqs. 5 and 6, define a maximum steady state efficiency that is the ratio of the entropy production in the load divided by the total entropy production (which is also the net input power): \(\eta_{\text{max}} \equiv \sigma_{\text{load}}/\sigma\). The current cancels, yielding an efficiency that is dependent only on elementary microscopic parameters, which are the resistances and the driving rate:

\[ \eta_{\text{max}} = \frac{\ln \left[ 1 + \left( \frac{R_{d,\text{Load}}}{R_d + R_{d,\text{Load}}} \right) \frac{\alpha_d}{k_d} \right]}{\ln \left( 1 + \frac{\alpha_d}{k_d} \right)}. \]

This efficiency limit holds for any singly-driven system, with all of the system-dependence absorbed into the single effective load resistance (Fig. 2a); there exists an infinite number of microstate circuits with equivalent \(R_{\text{load}}\).
Figure 2: Maximum efficiency of singly-driven process for arbitrary networks. Any equilibrium system can be driven by biasing a transition in the microstate circuit of the system’s phase space, thereby breaking detailed balance. The circuit can then be divided into the driven component, which is the resistance and voltage source of the driven transition, and the load component, which is the effective resistance (also called Thevenin resistance) due to the remainder of the circuit (a). Eq. 7 predicts the maximum achievable efficiency, defined as the ratio between the entropy production rate of the load and that of the entire circuit, and shows that maximum efficiency limits are system-sensitive near equilibrium but tend to unity far from equilibrium, as reflected in the typical concentration levels of the energy currency in living systems, ATP (b).

The efficiency of a system can be lower than the limit set in Eq. 7 due to system-dependent inefficiencies, such as futile cycles, within the load. The maximum efficiency is highly system-dependent near equilibrium, but universally approaches unity when driven far from equilibrium. In intracellular systems, \( \alpha_d \) corresponds to the rate of binding to a molecule of ATP; in vivo, the ATP concentration is kept above that of ADP despite the latter being about 60kJ/mol lower in energy: \( \alpha_d/k_d \approx 10^{10.4} \) [22]. At these concentrations, living systems are intrinsically capable of being nearly perfectly efficient over a wide range of load resistances corresponding to a wide range of cellular functions (Fig. 2b). This general and counter-intuitive prediction is consistent with the observation that isothermal ratchets and motors are more efficient in the strongly-driven regime [23-24].

2. Reciprocal Relations Far from Equilibrium

Another deep theorem of circuit theory is Lorentz reciprocity, which applies to circuits whose currents respond both linearly and symmetrically with the local potential. If the \( ij \) transition in a circuit is directly driven by a voltage source \( E_{ij} \), in addition to the local current response \( I_{ij} \), a nonlocal current would be indirectly induced between other pairs of neighboring states, say \( m \) and \( n \), denoted by \( I_{mn} \). Likewise, if a voltage source \( E_{mn} \) is placed between \( m \) and \( n \), then a reciprocal current \( I_{mn} \) would also be induced. The reciprocity theorem states that \( I_{mn} = I_{ij} \) if \( E_{mn} = E_{ij} \). Combining this theorem with Eq.4, \( E_{ij} = (R_{ij} + R_{ij, Load})I_{ij} \), yields the general reciprocal relation:

\[
I_{ij} = I_{mn} [\frac{R_{mn} + R_{mn, Load}}{R_{ij} + R_{ij, Load}} I_{ij}] \quad (8)
\]
which is valid arbitrarily far from equilibrium. The resistance ratio in Eq. 8 describes the linear asymmetry in current response when the local driven transition and the nonlocal measured current are swapped. Eq. 8 describes total response to driving, rather than perturbative response \[25\]. Remarkably, the reciprocal current relation remains linear even if the current response becomes nonlinearly related to the driven rate constant when the system is far from equilibrium.

Close to equilibrium, the induced current is approximately proportional to the potential (linear response): \[ I_{ij \rightarrow mn} = \gamma_{ij \rightarrow mn} \ln(1 + \alpha_{ij}/k_{ij}), \] with \( \gamma_{x \rightarrow y} \) being the linear susceptibility of the current response at \( y \) due to a voltage source placed at \( x \). In this case, the perturbed microstate probabilities are equal to their equilibrium values to first order in \( \alpha / k \), such that \[ \mathcal{E}_{ij} = (\alpha_{ij}/k_{ij})P_i^e/P_i^e \approx (\alpha_{ij}/k_{ij}). \] Eq. 8 then simplifies to Onsager’s reciprocal relations for microstates near equilibrium \[26\].

\[ \gamma_{mn \rightarrow ij} = \gamma_{ij \rightarrow mn}. \] (9)

Eq. 9 is valid for multiple driven sources because voltage sources are independent (non-voltage-dependent) near equilibrium. The general Eq. 8 applies when a single transition is driven at a time because of nonlinear feedback between multiple sources in the strongly-driven regime.

The reciprocal relation Eq. 8 allows for the engineering, via human design or evolution, of resistances and placement of driven transition in order to achieve a desired response to driving. In particular, there exists a transition \( ij \) in any equilibrium system that, if driven, induces a maximum asymmetric current response with respect to every other transition in the system, regardless of the extent of driving. This transition is the one with the maximum \( R_{ij} + R_{ij,\text{Load}} \) and is therefore the transition that the system is most “sensitive” to. It remains to be seen if biological processes have evolved such that ATP hydrolysis occurs at such maximally sensitive transitions within their phase space.

**IV. APPLICATION TO THE CYCLE TOPOLOGY**

Using the generalized Boltzmann distribution Eq. 4, we show here how current and probability distributions depend on the driving rates and resistances for the topologically simplest microstate circuits: work cycles, which are the most common circuits found in molecular biology, as well as building blocks of more complex systems.

1. **Singly Driven Cycles: Transforming Local to Global Stability**

Consider a system with a single driven transition (Fig. 3a). Without loss of generality, define the driven transition to be from state 1 to state 2. Then the microstates can be systematically combined into a single voltage source and single resistor \( R_{\text{tot}} = R_{12} + R_{12,\text{Load}} \). If the microstates are arranged along a single cycle, such as in the case of the work cycle for an enzyme or molecular motor, \( R_{\text{tot}} \) is simply the sum of all of the resistances (resistors in series). The current through the cycle is given by applying Eq. 4 to the loop: \[ I = \frac{1}{R_{\text{tot}}} \sum_i (\alpha_{ij}/k_{ij})(P_i^e/P_i^e); \] as before, the equilibrium probability is starred. The relative probability of any two microstates \( i \) and \( j \) is given by:

\[ P_i = P_i e^{\beta(G_j - G_i)} \left[ \frac{R_{\text{tot}} + \alpha_{12}/k_{12} R_{1j}}{R_{\text{tot}} + \alpha_{12}/k_{12} R_{1j}} \right], \] (10)

where \( R_{ij} \) is the sum of the resistances from \( i \) to \( j \) and the resistance ratio describes the deviation from equilibrium. In the highly driven limit, \( \alpha_{12} \gg k_{12} \), the driven microstate becomes negligibly occupied, and all other microstates have a relative probability of: \[ P_i \approx P_i e^{\beta(G_j - G_i)} R_{1j}/R_{1j} \]. If there is a large resistance between states \( i \) and \( j \), the relative probabilities of these states can be very different from the equilibrium value. In the high temperature limit (\( \beta G_i \ll 1 \)), the probability of microstate \( i \) is proportional to the sum of the reciprocal forward rate constants from \( i \) to 1, in contrast to the equilibrium condition in which all microstates are equally probable. The current is:
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Figure 3: Examples of single cycle circuit topologies. The singly driven cycle with arbitrary number of microstates with differing resistances (a), and the doubly driven 6-state cycle descriptive of protein (de)activation cycles (b), are considered as concrete examples of the application of the nonequilibrium Boltzmann equation.

\[
I = \frac{\frac{a_{12}}{k_{12}}}{R_{\text{tot}} + \sum_{i=1}^{N} R_{i1} P_i^*}, \quad (11)
\]

For small driving rates, the current has a linear response to the driven ratio \( \frac{a_{12}}{k_{12}} \), with the proportionality constant being the inverse of the total resistance. For large driving rates, the current approaches a plateau value: \( I_{\text{max}} = \sum_{i=1}^{N} R_{i1} P_i^* \). For systems in which the variability of the resistances are dominated by free energy differences rather than forward rates, the current at maximum driving is:

\[
I_{\text{max, bc}} \approx k_{\text{peak}} e^{-\beta (G_{\text{peak}} - G_{\text{valley}})}, \quad (12)
\]

In this barrier-crossing ("bc") scenario, the maximum current is set by the rate of leaving the highest free energy (peak) microstate, but exponentially suppressed by the free energy barrier. Such a temperature dependence has been experimentally measured for the rate of enzymatic activity [27]. However, two distinctions should be made that differentiates Eq. 12 from the classic Arrhenius interpretation. First, the rate prefactor here is the elementary rate of exiting the peak state rather than the attempt frequency at the valley state. Second, the exponent is the net free energy difference between the lowest free energy intermediate state (valley) and the peak, and does not necessarily correspond to the free energy difference between adjacent microstates along the cyclic pathway.

1.1 Three-state cycle

As a concrete example, consider the special case in which there are only three states, which is the minimum needed for a cycle, and is commonly used to model enzymatic reactions. In this case, the cycle transforms substrate, with concentration [S] into products with concentration [P], using enzymes with total concentration [Eo]. The system is maintained at steady state by constant replacement of products with reactants at a rate \( \alpha \) that drives state 1 (defined to be "enzyme + free product") to state 2 (defined to be "enzyme + free substrate"). Without the enzyme, the spontaneous interconversion rates between substrate and product are much smaller than the other rates. The rate constant of binding substrate to enzyme is proportional to [S] :

\[
k_{23} \equiv k_f [S]. \quad k_{\text{cat}} \equiv k_{31}
\]

Substituting into Eq.11 gives the expression for rate of product formation:

\[
v = \frac{k_{\text{cat}} [Eo] [S]}{K_M + [S]} \left( \frac{\alpha P^*_2}{\alpha P^*_2 + \frac{k_{\text{cat}} [S]}{K_M + [S]}} \right), \quad (13)
\]

where \( K_M \equiv (k_{\text{cat}} + k_f)/k_f \) and \( v = [Eo] I \). The systems becomes irreversibly driven if \( \alpha \) is much larger than the rate of spontaneous conversion from substrate back to product. In that case,
A ubiquitous biochemical process is the activation and inactivation of proteins via the chemical attachment and removal of a phosphate group to the protein. Proteins are phosphorylated and dephosphorylated by kinases and phosphatases, respectively, which are themselves also proteins. About one third of all proteins are esters, which are themselves also enzymes such as ATPases.

1.2 Six-state cycle

A ubiquitous biochemical process is the activation and inactivation of proteins via the chemical attachment and removal of a phosphate group to the protein. Proteins are phosphorylated and dephosphorylated by kinases and phosphatases, respectively, which are themselves also proteins. About one third of all proteins are esters, which are themselves also enzymes such as ATPases.

2. Doubly Driven Cycles: Achieving Tunability

Consider the biologically-relevant case in which both phosphorylation and dephosphorylation are directly driven. Since both driven rate constants are proportional to ATP concentration, for simplicity, assume that \( \alpha/k \equiv \alpha_{23}/k_{23} = \alpha_{56}/k_{56} \). Solving Eq. 4, the fraction of the protein in the on state can be calculated as a function of \( \alpha/k \). Close to equilibrium, and for the case when \( G_5 > G_2 \), this gives \( P_{on} \approx P_{on}^* (1 + \alpha/k(P_1^* + P_2^* + P_6^*)) \). Far from equilibrium, when \( \alpha/k \gg P_4^* \), define \( P_{max} \equiv e^{-\mu_{bind} k_{4}} \), which is the equilibrium probability of state 4 if all other states except state 3 were forbidden. Then,

\[
P_{on} = \frac{1}{\mu_{bind} k_{4}} + \left( \frac{1}{k_{5}^*} + \frac{1}{k_{3}} + \frac{1}{k_{6}} \right) k_{4} \equiv \frac{1}{\mu_{bind} k_{4}} \Rightarrow \text{for simplicity, assume that } \alpha/k \equiv \alpha_{23}/k_{23} = \alpha_{56}/k_{56} \.)
\]

where \( \mu_{bind} \) is the ratio of the phosphatase binding rate to un-phosphorylated versus phosphorylated protein. If kinase concentration is kept fixed, all of these parameters are constants except for \( k_4 \), which is proportional to the phosphatase concentration.

The switching off of the protein (i.e. depleting the "on" state 4) can be accomplished by decreasing the phosphatase concentration, and is either dominated by the linear term (non-cooperative) or quadratic term (cooperative) in the denominator of Eq. 14, depending on the values of the other rate constants. In either case, the doubly-driven circuit is capable of turning on and off the active state of MAPK by changing the concentration of the phosphatase (Fig. 4e). By symmetry, the kinase concentration could also be used to turn MAPK on or off. Having two driven transitions is sufficient to stabilize an otherwise unstable functional microstate, as well.
as to control its stability in response to changing needs by modifying the resistances.

V. DISCUSSION

Since the time of Gibbs, the free energy has been recognized as the microscopic determinant of system behavior at equilibrium. This work shows the Boltzmann distribution to be the special case of a more general distribution which holds regardless of how far a system is from equilibrium. The generalized equation allows for the calculation of probabilities and currents, and is isomorphic to a resistor circuit with voltage-dependent voltage sources. These resistors can be systematically coarse-grained into effective variables, enabling lossless simplification. Out of equilibrium, when currents are nonzero, the concept of the resistance is equally as important as that of the energy. This mapping allows for the cross-application of general circuit theorems to nonequilibrium statistical mechanics to yield the reciprocal and maximum efficiency relations, which hold for all systems driven arbitrarily far from equilibrium by an energy source.

The nonequilibrium Boltzmann equation and its associated formalism and theorems are well-suited to probe living systems, which consist of a sparse subset of transitions (ATP binding) that are directly driven far from equilibrium, thereby breaking detailed balance for the entire interconnected phase space. The theory leads to testable predictions relating observables ($I_{ij} \text{ and } P_i$) to both macroscopic ($\alpha_{\text{ATP}}$ and $T$) and microscopic parameters ($k_{ij}$ and $G_i$), which can be independently confirmed in calorimetry, time-resolved-spectroscopy, and/or mutagenesis experiments. This approach offers a concrete way to answer general questions about living systems, such as whether high efficiency is intrinsic or due to evolutionary fine-tuning, how cell fate can be shielded from some biochemical perturbations while being sensitive to others, and how complexity coexists with stability and tunability.

Figure 4: Separating function from stability in a phosphorylation cycle. The 6-state microstate cycle (a) has two driven transitions. Without driving, the "on" state 4 is negligible (b), but becomes globally stable when the phosphorylation transition is driven (Driven 1; c). When both phosphorylation and dephosphorylation is driven, the "on" state can be turned (d) on as well as off by changing phosphatase concentration, the latter of which is not achievable with a single driven transition (e). Parameters are from Ref. [30]
In the future, it would be fruitful to use this formalism to investigate the nonlinear interplay of multiple driven sources to produce emergent behaviors such as periodic and chaotic dynamics. It is also hoped that the circuit approach will allow, as in electrical engineering, the design of artificial micro- and nanoscopic machines that perform as well as those in nature.

VI. Methods

For the 6-state (de)phosphorylation model, ATP hydrolysis-driven (de)phosphorylation is lumped into the ATP-binding step because the former occurs irreversibly and much faster than the latter step under physiological conditions. State 4 corresponds to the detachment of the kinase from the phosphorylated protein and is therefore the “on” state of the protein. The coefficient $k_1$ is the kinase binding rate constant to un-phosphorylated protein, $k_3$ is the kinase un-binding rate constant from the phosphorylated protein, $k_6$ is the phosphatase unbinding rate constant from the un-phosphorylated protein, and $k_4$ is the kinase binding rate constant to phosphorylated protein. Note that $k_1$ and $k_4$ are proportional to unbound kinase and phosphatase concentrations, respectively, and that the free energies are dependent on the concentrations via the change in entropy upon kinase or phosphatase binding. The microstate parameters are taken from table S1 of [30].

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