Reversal symmetries for cyclic paths away from thermodynamic equilibrium

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Abstract

If a system is at thermodynamic equilibrium, an observer cannot tell whether a film of it is being played forward or in reverse: any transition will occur with the same frequency in the forward as in the reverse direction. This principle of detailed balance arises from the fundamental time-reversal symmetry of physical laws. However, if expenditure of energy perturbs the rate of even a single transition from its equilibrium value, the symmetry undergoes a widespread breakdown, far beyond the point at which the energy is expended. Energy expenditure also leads to a combinatorial explosion of interdependency, with steady-state probabilities of system states depending in a complicated manner on the rate of every transition in the system. Nevertheless, in the midst of this non-equilibrium complexity, we find that cyclic paths have reversibility properties that remain local, and which can exhibit symmetry, no matter how far the system is from thermodynamic equilibrium. Specifically, given any cycle of reversible transitions, the ratio of the frequencies with which the cycle is traversed in one direction versus the other is determined, in the long-time limit, by the thermodynamic force associated with the cycle itself, without requiring knowledge of the rest of the system. In particular, if energy is expended elsewhere but not on the cycle itself, then, over long times, the cycle traversal frequencies are the same in either direction and exhibit time-reversal symmetry.

INTRODUCTION

To demonstrate our results we use the “linear framework”, a graph-based approach to Markov processes, which was introduced to analyze biochemical systems under timescale separation [1]. Aspects of this graph-based approach have appeared previously in the physics literature, in the work of Hill [2] and Schnakenberg [3], and we hope the results presented will reinforce the significance of their pioneering insights. The graph provides the mathematical setting in which the substantial difference in parametric complexity between equilibrium and non-equilibrium states becomes clearly visible. In this context, we rigorously demonstrate the unexpected simplicity and symmetry in the properties of cycles under time-reversal, even in systems that are far from equilibrium. As the linear framework has been described in several publications, we outline here only what is needed to set our results in context; for more background and history, see [1, 4]; for further details and relevant applications, see [5, 6, 7, 8]; for reviews, see [9, 10].

A linear framework graph, $G$, is a finite, directed graph with labeled edges and no self-loops. For the purposes discussed here, the vertices, denoted $1, \cdots, n$, represent the states in which one might find a given realization of the system. We call them “mesostates” to emphasize that while they refer to the configuration of the system and not to an ensemble, they are not microstates in the customary physical sense. The edges, denoted $i \rightarrow j$, represent transitions between mesostates; and the edge labels, denoted $\ell(i \rightarrow j)$, represent infinitesimal transition rates for an underlying Markov process and are positive quantities with dimensions of $[\text{time}]^{-1}$. If we let $X(t)$ denote the underlying Markov process at time $t$, then this process is specified by a conditional probability distribution $\Pr(X(t) = i \mid X(s) = j)$, where $i$ and $j$ are mesostates in $G$ and $s < t$. With this notation,

$$\ell(i \rightarrow j) = \lim_{\Delta t \to 0} \frac{\Pr(X(t + \Delta t) = j \mid X(t) = i)}{\Delta t}. \tag{1}$$

Edge labels may include expressions which specify the interaction between mesostates and entities in the environment of the graph, such as reservoirs of particles or thermal energy, and these expressions must be determined in any specific application of the framework [10]. For our purposes here, as we will explain below, we can consider the labels to be symbolic constants representing positive real numbers.

The master equation of the Markov process describes the time evolution of mesostate probabilities, described by the column vector, $\mathbf{p}(t)$, where $p_i(t)$ is the probability that the system is in mesostate $i$ at time $t$. This master equation can be obtained from $G$ as,

$$\frac{dp_i}{dt} = \sum_{j \neq i} (p_j \ell(j \rightarrow i) - p_i \ell(i \rightarrow j)).$$
Note that, since each edge has only one source vertex, each $dp_i/dt$ is linear in the $p$’s. We can therefore represent the master equation as a linear differential equation,
\[
\frac{dp}{dt} = L(G) \cdot p(t),
\] (2)
where $L(G)$ is the $n \times n$ Laplacian matrix of $G$ [H] and $p$ is the column vector of mesostate probabilities. It is easy to see that the column sums of $L(G)$ are zero, which corresponds to the conservation of total probability. The relationship described above between graphs and Markov processes is quite general: given any continuous-time, finite-state Markov process, for which infinitesimal rates can be defined, there is a graph $G$ for which Eq. 2 specifies the master equation [4].

For the applications considered here, we will assume that any changes in the transition rates take place slowly compared to the time taken for $p(t)$ to reach steady state, so that the edge labels can be treated as constants.

If the system is in contact with a single thermal reservoir at temperature $\beta^{-1}$ and/or one or more particle reservoirs at the same chemical potential, $\mu$, it will reach thermodynamic equilibrium. On the other hand, the system may be maintained away from equilibrium. This could happen through contact with multiple reservoirs at different temperatures or chemical potentials. The latter is common in biological systems, from molecular motors which exploit a chemical potential difference between ATP, ADP and inorganic phosphate to enzymes which operate in the presence of a chemical potential difference between substrate and product. Alternatively, as in the case of active matter, chemical or other energy may be consumed by the system itself, and then dissipated to the environment. Either kind of non-equilibrium system is amenable to a graphical treatment within the linear framework.

This graph-based description of the Markov process allows effective calculation of quantities of interest whether or not the system is at thermodynamic equilibrium (Eqs.3 and 4 below). We outline the details of how this is done in the next section before stating and proving our main results.

RESULTS

Departure from equilibrium in the linear framework

Steady states and the Matrix-Tree theorem

We will assume from now on that $G$ is strongly connected, so that any pair of vertices, $i, j$, are connected by a directed path of contiguous edges, $i = i_1 \to \cdots \to i_k = j$. In this case, Eq. 2 has a unique steady state, $p^*$. Equivalently, the kernel of the Laplacian matrix is one-dimensional, $\dim \ker L(G) = 1$. A canonical basis element, $\rho(G) \in \ker L(G)$, can be calculated from $G$ through the Matrix-Tree theorem (MTT),
\[
\rho_i(G) = \sum_{T \in \Theta_i(G)} \left( \prod_{j<k \in T} a \right).
\] (3)

Here, $\Theta_i(G)$ is the set of spanning trees of $G$ rooted at vertex $i$. A spanning tree is a subgraph of $G$ which includes all vertices of $G$ (spanning) and has no cycles when edge directions are ignored (tree). It is rooted at $i$ if $i$ is the only vertex with no outgoing edges. Since $p^* = \lambda \rho(G)$, for some scalar $\lambda \in \mathbb{R}^+$, it follows from the conservation of probability that,
\[
p_i^* = \frac{\rho_i(G)}{\rho_1(G) + \cdots + \rho_n(G)}.
\] (4)

Eq. 4 expresses steady-state probabilities in terms of the edge labels, through Eq. 3 and can be applied to a system maintained arbitrarily far from thermodynamic equilibrium. The MTT is not required for the main result below but, in conjunction with Eq. 7 below, it illustrates the dramatic increase in parametric complexity that arises away from equilibrium. Eq. 3 shows that the steady state probability of each mesostate becomes dependent in a complicated way on edge labels throughout the graph. The MTT in Eq. 3 was known to Hill [2] and Schnakenberg [3] but then seems to have disappeared from view; for its convoluted history across many disciplines and a proof, see [4].

Thermodynamic equilibrium

If a system can reach thermodynamic equilibrium, then it must obey the principle of detailed balance. This principle holds from elementary to mesoscopic systems: observing a trajectory over time, transitions from configuration $i$ to $j$ and from configuration $j$ to $i$ will happen with equal frequency, however coarse- or fine-grained the conceptions of $i$ and $j$ may be [12]. Detailed balance arises from the fundamental time-reversal symmetries in nature [13].

For a graph to represent a system that obeys detailed balance, it must first of all be reversible: if there exists $i \to j$, then there is an edge $j \to i$, which is the reverse transition (and not merely an alternative way of returning from $j$ to $i$).
Note that a reversible graph is strongly connected if, and only if, it is connected. Second, for any pair of reversible edges, $i = j$,

$$p_i^* \ell(i \to j) = p_j^* \ell(j \to i).$$

(5)

The “probability flux” along an edge $i \to j$ is given by $J_{i \to j} = p_i^* \ell(i \to j)$. Eq. 5 says that, at thermodynamic equilibrium, the probability flux at each pair of reversible edges is in balance, irrespective of any other transitions in which the mesostates are engaged. We therefore refer to this equilibrium property as flux balance. Eq. 4 reveals that, at thermodynamic equilibrium, it is the ratio of the edge labels which is the essential parameter. The link to equilibrium statistical mechanics arises from the existence of a free energy, $F$, associated to each mesostate $i$, which determines the label ratio

$$\frac{\ell(i \to j)}{\ell(j \to i)} = e^{\beta(F_i - F_j)}.$$  

(6)

Eq. 5 leads to an alternative basis element $\mu(G) \in \ker \mathcal{L}(G)$. Choose a reference mesostate in $G$, usually taken to be the mesostate indexed by 1. Given any path of reversible edges from 1 to a given mesostate $i$, $1 = i_1 \equiv i_2 \equiv \cdots \equiv i_{k-1} \equiv i_k = i$, let

$$\mu_i(G) = \frac{\ell(1 \to i_1)}{\ell(i_2 \to i_1)} \cdots \frac{\ell(i_k \to i_{k-1})}{\ell(1 \to i_1)}.$$  

(7)

It is clear from Eq. 5 that $\mu_i(G)$ does not depend on the chosen path from 1 to $i$ and that, because $\dim \ker \mathcal{L}(G) = 1$ and $\mu_1(G) = 1$, $p_i^* \mu(G) = p_i^*$. It follows that $\mu(G) \in \ker \mathcal{L}(G)$ and that $\rho_1(G) \mu(G) = \rho(G)$. Substituting this relationship into Eq. 4 and using Eq. 6 recovers the classical formula of equilibrium statistical mechanics for the steady-state probability of a mesostate. The denominator of Eq. 4 is then proportional to the partition function but we see from this treatment that the denominator of Eq. 4 also provides an analog of the partition function for a non-equilibrium system.

**Non-equilibrium complexity and the breakdown of flux balance**

The comparison between $\mu(G)$, at thermodynamic equilibrium, and $\rho(G)$, away from equilibrium, is instructive. With the former, Eq. 7 shows that it is sufficient to take any single path in $G$ to a given mesostate to calculate its steady-state probability using Eq. 4. With the latter, when detailed balance is broken, not only do steady-state probabilities become path-dependent, every path in $G$ makes a contribution to them and the MTT in Eq. 3 provides the bookkeeping for this calculation. The combinatorial explosion coming from enumerating all rooted spanning trees can be super-exponential in the size of $G$ and the steady-state probability of a mesostate can come to depend in an extremely complicated manner on all the labels in $G$. The graph-based approach here provides a vivid demonstration of the profound difference between equilibrium and non-equilibrium states.

The impact of non-equilibrium path-dependency is felt globally even if energy expenditure is limited and local. If we take a graph $G$ that obeys detailed balance, and perturb even a single edge label, $\ell(i \to j)$, so as to break detailed balance, the resulting change in $p^*$ due to Eq. 3 leads to a widespread breakdown of flux balance (Eq. 5) even at edges whose labels retain their equilibrium values.

**Cycles and thermodynamic forces**

An equivalent condition to the statement of detailed balance in Eq. 5 can be given in terms only of the edge labels. Consider any cycle of reversible edges, $i_1 \equiv i_2 \equiv \cdots \equiv i_{m-1} \equiv i_m = i_1$. Cycles need not be simple; the same vertex may appear more than once, with more than one index, in the numbered list. At thermodynamic equilibrium, the product of the labels in one direction around the cycle equals the product in the other direction,

$$\ell(i_1 \to i_2) \cdots \ell(i_{m-1} \to i_m) = \ell(i_m \to i_{m-1}) \cdots \ell(i_2 \to i_1).$$

(8)

Eq. 8 makes clear that, if $G$ can reach thermodynamic equilibrium, the edge labels are not independent quantities. However, the algebraic relationships between edge labels can be fully determined by “exchange formulas” 5. If a graph is at thermodynamic equilibrium, flux balance as given by Eq. 5 and the cycle condition as given by Eq. 8 are equivalent statements (for a proof, see the SI of Ref. [8]).

Let $C$ denote any cycle of reversible edges, $C : i_1 \equiv i_2 \equiv \cdots \equiv i_{m-1} \equiv i_m = i_1$. The thermodynamic force associated with the cycle, $\tilde{A}(C)$, is defined as the logarithm of the ratio of the corresponding terms in Eq. 8

$$\tilde{A}(C) = \log \left( \frac{\ell(i_1 \to i_2) \cdots \ell(i_{m-1} \to i_m)}{\ell(i_m \to i_{m-1}) \cdots \ell(i_2 \to i_1)} \right).$$

(9)

It follows from Eq. 8 that for a system at thermodynamic equilibrium $\tilde{A}(C) = 0$ for all cycles. Schnakenberg used the thermodynamic force in Eq. 9 along with the net flux across a reversible edge, $\dot{J}_{ij} = J_{i \to j} - J_{j \to i}$, to calculate the entropy production rate in a non-equilibrium steady state 3.
As we have defined it, a cycle specifies its starting mesostate, but properties like those above are independent of the starting point. The following notation will therefore be helpful. Given a cycle $C : i_1 \rightleftharpoons i_2 \rightleftharpoons \cdots \rightleftharpoons i_{m-1} \rightleftharpoons i_m = i_1$, let $C(i_j)$, where $1 \leq j \leq m$, denote the cycle with the same order of transitions starting at mesostate $i_j$, $C(i_j) : i_j \rightleftharpoons i_{j+1} \rightleftharpoons \cdots \rightleftharpoons i_{m-1} \rightleftharpoons i_1 \rightleftharpoons i_2 \rightleftharpoons \cdots \rightleftharpoons i_{j-1} \rightleftharpoons i_j$. It follows that $C(i_1) = C(i_m) = C$. Let $[C]$ denote the set of cycles having the same cyclic order of transitions, $[C] = \{C(i_1), C(i_2), \ldots, C(i_{m-1})\}$. Note that the cycle condition in Eq. 8 and the thermodynamic force in Eq. 9 are both well-defined as properties of $[C]$.

**Main result: reversibility properties of cyclic paths**

We need some further notation to state the main result. Let $P : i_1 \rightleftharpoons i_2 \rightleftharpoons \cdots \rightleftharpoons i_{m-1} \rightleftharpoons i_m$ be any path of reversible edges in a graph $G$. $P$ may be a single microstate with $m = 1$ or a cycle with $i_1 = i_m$. Consider observing a realisation, or trajectory, of the underlying Markov process as transitions occur between mesostates and denote the number of times that the path $P$ is observed up to time $t$ by $n(P,t)$. It is important that all the mesostates on the path are observed in the specified order without deviations. By $\Pr(P)$, we denote the probability that the Markov process, having started in state $i_1$, will subsequently go through the transitions in $P$ in exactly the specified order without deviation. Since $G$ is strongly connected with positive labels, we can appeal to the ergodic theorem for Markov processes [11] to interpret the probability in frequentist terms,

$$\Pr(P) = \lim_{t \to \infty} \frac{n(P,t)}{n(i_1,t)},$$

where the denominator is the number of times in which the starting mesostate, $i_1$, is observed along the trajectory.

Given a cycle $C : i_1 \rightleftharpoons i_2 \rightleftharpoons \cdots \rightleftharpoons i_{m-1} \rightleftharpoons i_m = i_1$ let $C'$ denote the reverse cycle, $C' : i_m \rightleftharpoons i_{m-1} \rightleftharpoons \cdots \rightleftharpoons i_2 \rightleftharpoons i_1 = i_m$. We can easily extend the definition to cycle sets by defining $[C]' = [C']$. Along a trajectory, the same cyclic order of transitions may be observed starting at any mesostate, so we can define $n([C],t)$ to be,

$$n([C],t) = n(C(i_1),t) + \cdots + n(C(i_{m-1}),t).$$

**Theorem:** For any connected reversible graph, any cycle $C : i_1 \rightleftharpoons i_2 \rightleftharpoons \cdots \rightleftharpoons i_{m-1} \rightleftharpoons i_m = i_1$ of reversible edges in $G$ and any mesostate $i_j$ on $C$,

$$\lim_{t \to \infty} \frac{n(C(i_j),t)}{n(C(i_j)',t)} = \lim_{t \to \infty} \frac{n([C],t)}{n([C]',t)} = e^{\hat{A}(C)}.$$

The proof of this requires a couple of steps.

**Proposition:** For any connected reversible graph, any cycle $C : i_1 \rightleftharpoons i_2 \rightleftharpoons \cdots \rightleftharpoons i_{m-1} \rightleftharpoons i_m = i_1$ of reversible edges in $G$ and any mesostate $i_j$ on $C$,

$$\frac{\Pr(C(i_j))}{\Pr(C(i_j)')} = e^{\hat{A}(C)}.$$

**Proof:** Conditional on the system being in mesostate $i_1$, the probability that its next transition will be to state $i_2$ is given by

$$\ell(i_1 \to i_2) \sum_{i \to j} \ell(i_1 \to j),$$

where the sum in the denominator is over all outgoing edges in $G$ from $i_1$. Let us denote this latter quantity, for a given mesostate $i$, by $Z(i) = \sum_{i \to j} \ell(i \to j)$, so that $Z(i_1)$ gives the denominator in Eq. 11. Since the probability in Eq. 11 leads to mesostate $i_2$, the calculation can be continued to determine the probability, conditional on starting in mesostate $i_1$, that the system makes the transition from $i_1$ to $i_2$ followed by the transition from $i_2$ to $i_3$,

$$\frac{\ell(i_1 \to i_2)}{Z(i_1)} \cdot \frac{\ell(i_2 \to i_3)}{Z(i_2)}.$$

It follows by induction that, conditional on starting in mesostate $i_2$, the probability that the next $m - 1$ transitions will be as specified by cycle $C$ is given by,

$$\prod_{k=1}^{m-1} \ell(i_k \to i_{k+1}) \prod_{k=1}^{m-1} Z(i_k).$$
The equivalent expression for \( C' \) is given by,
\[
\prod_{k=2}^{m} \frac{\ell(i_k \to i_{k-1})}{Z(i_k)}.
\]  
(14)
Hence, taking the ratio of Eq. 13 by Eq. 14 and recalling that \( i_1 = i_m \) and the definition of thermodynamic force in Eq. 9, we see that,
\[
\frac{\Pr(C)}{\Pr(C')} = \left( \frac{Z(i_m)}{Z(i_1)} \right) \prod_{k=1}^{m-1} \frac{\ell(i_k \to k_{k+1})}{m \prod_{k=2}^{m} \ell(i_k \to i_{k-1})} = e^{A(C)}.
\]  
This final value is independent of the starting mesostate \( i_1 \), from which the result follows.

Q.E.D.

It follows from Eq. 10 that \( \Pr(C(i_j)) = \lim_{t \to \infty} n(C(i_j), t)/n(i_j, t) \) and \( \Pr(C(i_j)'') = \lim_{t \to \infty} n(C(i_j)'', t)/n(i_j, t) \). By the Proposition, both of these quantities are non-zero and, therefore,
\[
\lim_{t \to \infty} \frac{n(C(i_j), t)}{n(C(i_j)'', t)} = \lim_{t \to \infty} \frac{n(C(i_j), t)/n(i_j, t)}{n(C(i_j)'', t)/n(i_j, t)} = \frac{\Pr(C(i_j))}{\Pr(C(i_j)'')} = e^{A(C)},
\]  
(16)
which establishes part of the Theorem. The remaining part comes from the following observation.

Lemma: If functions \( a_j(t) \) and \( b_j(t) \) are defined for \( 1 \leq j \leq m \) and satisfy \( \lim_{t \to \infty} a_j(t)/b_j(t) = \alpha \) for each \( j \), then
\[
\lim_{t \to \infty} \frac{a_1(t) + \cdots + a_m(t)}{b_1(t) + \cdots + b_m(t)} = \alpha.
\]
Proof: It is sufficient to take \( m = 2 \), since the general case follows by induction. Choose \( \epsilon > 0 \) and take \( t \) sufficiently large that \( \left| \frac{a_1(t) - \alpha b_1(t)}{b_1(t)} \right| < \epsilon/2 \) and \( \left| \frac{a_2(t) - \alpha b_2(t)}{b_2(t)} \right| < \epsilon/2 \), which we may do by hypothesis. Dropping \( t \) to simplify the syntax,
\[
\left| \frac{a_1 + a_2}{b_1 + b_2} - \alpha \right| = \left| \left( \frac{a_1 - \alpha b_1}{b_1} \right) \left( \frac{b_1}{b_1 + b_2} \right) + \left( \frac{a_2 - \alpha b_2}{b_2} \right) \left( \frac{b_2}{b_1 + b_2} \right) \right| < \epsilon,
\]
from which the result follows.

Q.E.D.

Taking \( a_j(t) = n(C(i_j), t) \) and \( b_j(t) = n(C(i_j)'', t) \) and using Eq. 16 completes the proof of the Theorem.

Time reversal symmetry

In the event that energy is expended elsewhere than on the cycle \( C \), so that the thermodynamic force on the cycle is zero, \( \hat{A}(C) = 0 \), the path-reversal symmetry in the Theorem implies a time-reversal symmetry. Time reversal of a graph \( G \) yields a graph \( G^* \) such that \( \ell_G(i \to j) = \ell_G(j \to i) \). This means that \( \Pr(C(i_j)) \) on \( G \) is equal to \( \Pr(C(i_j)'') \) on \( G^* \). Hence, if \( \hat{A}(C) = 0 \), then the Theorem tells us that \( \lim_{t \to \infty} \frac{n(C(i_j), t)}{n(C(i_j)'', t)} \) is invariant under time reversal.

It is worth contrasting what happens to cycles of reversible edges away from equilibrium, as described in the Theorem, with the breakdown of detailed balance for any single pair of reversible edges, \( i \leftrightarrow j \). If an observer counts the transitions \( i \to j \) and \( j \to i \) along a trajectory of the system, then, appealing again to the ergodic theorem for Markov processes [11], the flux balance specified by Eq. 5 at thermodynamic equilibrium has the frequentist interpretation,
\[
\lim_{t \to \infty} \frac{n(i \to j, t)}{n(j \to i, t)} = 1.
\]  
(17)
By what was said in the previous paragraph, the ratio in Eq. 17 is preserved under time reversal. However, away from thermodynamic equilibrium, Eq. 17 no longer holds. Indeed, in this situation, by virtue of the MTT in Eq. 3, the ratio in Eq. 17 depends in a complicated way on all the edge labels in the graph, irrespective of where in the graph energy is being expended, and is no longer time-reversal symmetric.
**DISCUSSION**

The principle of detailed balance at equilibrium is stated succinctly by Dirk ter Haar: “...at equilibrium the number of processes destroying situation A and creating situation B will be equal to the number of processes producing A and destroying situation B” [12]. Hence, the observation of any process happening more frequently than its reverse is confirmation that the system is away from equilibrium. However, the breakdown of reversal symmetry in a non-equilibrium system is so pervasive that a single transition that is driven away from equilibrium can disrupt the balance between every pair of forward and reverse transitions and can make the steady-state probability of every system state dependent on all the rates in the system. Notwithstanding this path-dependent complexity, cyclic paths retain a remarkable symmetry under path reversal, as well as symmetry under time reversal, as long as none of the transitions in the cycle itself are driven away from equilibrium. Furthermore, the extent to which reversal symmetry is broken for a cycle which is driven away from equilibrium, as measured by the reversibility ratio in the Theorem, is a function only of the thermodynamic force on that cycle, irrespective of other thermodynamic forces acting elsewhere in the system.

Fig.1 illustrates the properties of cyclic paths for a simple graph derived from the biological phenomenon of kinetic cooperativity, or “allokairy” [14]. The mesostates are states of an enzyme with two conformations, which converts substrate $S$ into product $P$ only in its active conformation, $E$. The graph is coupled through its labels to two particle reservoirs, for $S$ and $P$. If the corresponding chemical potentials are equal (Fig.1B, top, with edge labels given in Fig.1C) the system is at thermodynamic equilibrium. In this case, the symmetry between each cyclic path and its reverse is preserved. If a chemical potential difference is now introduced on the product-rebinding edge, $E \rightarrow EP$, equilibrium is broken and the system becomes non-equilibrium. The red and blue cycles, which both include the edge $E \rightarrow EP$, no longer exhibit reversal symmetry, and their reversibility ratios approach the quantity given by Schnakenberg’s thermodynamic cycle force $A(C)$ (Eq. 9). (The force is easily seen to be the same for both cycles). In marked contrast, reversal symmetry is preserved for the red cycle, on which the edge $E \rightarrow EP$ is not present. This is true despite the green cycle sharing all but one of its reversible edge pairs with the blue cycle and despite non-zero net probability flux on each pair of reversible edges.

As Hopfield first showed for the case of error correction in the synthesis of biological molecules, thermodynamic equilibrium imposes fundamental limits—we have called them Hopfield barriers [5]—on the functional capacities of biological systems [15]. These limits that can be exceeded only by the expenditure of energy. The need to account explicitly for the non-equilibrium nature of biological processes has been felt even more acutely in recent years, and understanding the implications of the breakdown of detailed balance has been central to this effort [5, 8, 17]. A study by Battle et al. reported direct observations of detailed-balance violations in the dynamics of bacterial flagella [18]. Advances in single-molecule
experimental techniques are making it possible to observe in real time such processes as the conformational and binding behavior of enzymes [19], the activity of molecular motors [20], and the binding and unbinding of transcription factors during gene regulation [21]. These developments hold out the tantalising possibility that the results presented here could be used not only to confirm departure from thermodynamic equilibrium but also to identify the sources and quantify the extent of energy expenditure.

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