

Nonequilibrium theory for molecular machine design

Ying-Jen Yang ^{*}*Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, New York 11794, USA*Ken A. Dill *Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, New York 11794, USA;
Department of Physics and Astronomy, Stony Brook University, Stony Brook, New York 11794, USA;
and Department of Chemistry, Stony Brook University, Stony Brook, New York 11794, USA*

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Modeling biomolecular machines with the probability flows on Markov networks often entails computing node probabilities and edge fluxes with master equations and correlating machine performance with entropy production. However, this alone is not sufficient for design, optimization, and evolution because it does not treat cost-benefit trade-offs, topological misflows (backsteps, futile cycles, ineffective actions), or differential properties for flow design. Here, we develop CFT Design, based on the recently developed caliber force theory (CFT). We apply it to designing faster molecular motors through “traffic control”; optimizing speed, energy, and accuracy in kinetic proofreaders simultaneously to break their trade-offs; and designing better enzyme inhibitors based on their network topologies. CFT Design provides a general framework for optimizing nonequilibrium flows.

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I. INTRODUCTION: NETWORK FLOWS NEED DESIGN PRINCIPLES

The cyclic processes of molecular machines and enzymes underlie various dynamical functions in biology, e.g., energy transduction, motors and pumps, error correction, and catalysis of chemical reactions. Their mechanistic cycles can often be modeled as coarse-grained, Markov jump processes on discrete state networks [1–3]. In these models, transitions are assumed driven by thermal noise-driven escapes from energy wells. Hence, the transition rates k_{ij} are often treated with Arrhenius kinetics [4]. Rates and node probability distribution on these networks are often computed using master equations, focusing on correlating machine performance with the level of broken detailed balance quantified by entropy production [5–13].

What is missing are theoretical tools for optimizing and designing these flow networks. First, we need more general and systematic ways to treat the operational costs and benefits of flows. Whereas the costs and benefits in equilibrium (EQ) thermodynamics are often just heat and work, a much broader range of costs and benefits accrue in dynamical networks: useful products or advantageous flows versus waste or nonproductive flows. For example, biological copying

machines such as polymerases and ribosomes achieve balances of speed versus error versus energy costs. Moreover, dynamical costs and benefits depend not only on the rates of individual steps, but also on topological factors, traps, misflows, and slowdowns due to accidental wrong turns, futile cycles, or dead ends [14–16]. The present work gives a framework for systematically analyzing and optimizing the distributions and flows for performance metrics.

Second, nonequilibrium (NEQ) physics has lacked the powerful mathematical relationships that EQ thermodynamics uses to connect observables to conjugate forces under constraints—e.g., mapping (U, V, N) to $(1/T, p/T, -\mu/T)$ and to derive ensemble coordinates as well as fluctuation-response relations useful for design.

Here, we recast the optimization and control of NEQ molecular machines in terms of probability flows and formalize a design framework by integrating and extending recent developments in caliber force theory (CFT). CFT mirrors the conjugate structure of EQ thermodynamics to NEQ dynamics [17] and unifies fluctuation-response relations [18]. In this paper, we show that the primary utility of these theoretical foundations is to form a dual tool kit for NEQ flow design, which we call *CFT Design* (Fig. 1). The conjugate force-observable coordinates in Ref. [17] enable *inverse design*—solving for the transition rates required to achieve target probability distributions and flux performances under constraints (such as fixed chemical energy sources). Complementarily, CFT fluctuation-response relations in Refs. [17, 18] are useful for *forward design*—establishing how distributions and flow patterns are controlled by tuning specific kinetic parameters. We apply CFT Design to three biomolecular machines featuring distinct types of misflows to gain design

^{*}Contact author: ying-jen.yang@stonybrook.edu

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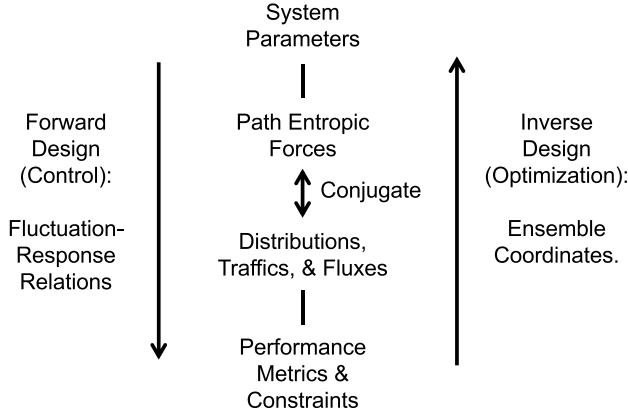


FIG. 1. The CFT Design framework. CFT serves as the theoretical bridge connecting system parameters (such as the transition rates of Markov jump processes) to performance metrics (such as speed, accuracy, or energetic cost). This paper shows its utility for forward design (routing flows via fluctuation-response relations) and inverse design (solving for the rates that reach target performances, including constrained optima, via ensemble coordinates).

insights into NEQ molecular machines: correcting backstepping in the F_1 -ATPase motor [19]; optimizing branching across incorporation and correction cycles in kinetic proof-readers [20–23]; and strengthening dead ends versus leaky loops in enzyme inhibition [24–26], which requires extending CFT to networks with noninvertible transitions.

II. THEORY: CALIBER FORCES DICTATE FLOW DESIGN

To begin, we first summarize the relevant parts of CFT, a principled general treatment of nonequilibria. Consider a molecular machine modeled as a Markov jump process (continuous-time Markov chain) on a finite network state space [e.g., simple graph like Fig. 2(a) or multigraph like Fig. 3(b)]. As illustrated in Fig. 2(b), the machine's long-term functional performance is characterized by its steady-state statistics, governed by three types of linearly independent observables [17]: the node probabilities π_n (the label n excludes an arbitrarily chosen reference node m to handle normalization), the symmetric edge traffics [16] $\tau_{ij} = \pi_i k_{ij} + \pi_j k_{ji}$, where k_{ij} is the transition rate from state i to j , and the antisymmetric edge net fluxes $J_{ij} = \pi_i k_{ij} - \pi_j k_{ji}$ spanned by the linearly independent basis of fundamental cycle fluxes J_c [27]. The set (π_n, τ_{ij}, J_c) forms a complete coordinate for the space of Markov dynamics as each k_{ij} can be parametrized as $(\tau_{ij} + J_{ij})/(2\pi_i)$. The goal is to control and optimize these observables by exploring the parameter space of transition rates (k_{ij}) .

For molecular machines driven by two or more fast-equilibrating reservoirs (assumed to be always at EQ), many transitions bind or release molecules from these reservoirs, such as ATP, substrates, or inhibitors. We model a single machine and hold the reservoir concentrations fixed. By mass action, a binding step is then pseudo-first-order, $k_{ij} = c_{ij} k_{ij}^0$. Here, k_{ij}^0 is the machine's own rate constant, and c_{ij} is the product of the concentrations of the molecules bound in that step, each relative to a standard concentration c° (e.g., $c_{ij} =$

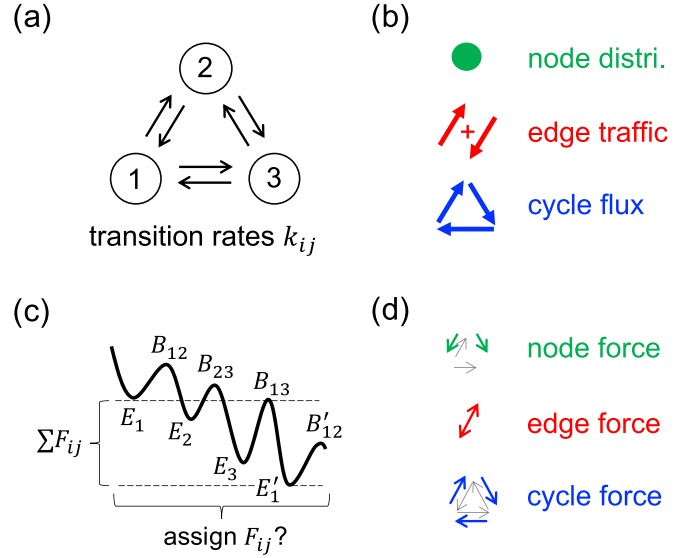


FIG. 2. A simple three-state machine process, with CFT properties defined. (a) A Markov network. (b) The observables: node probability distributions, symmetric edge traffics, and directed cycle fluxes. (c) Commonly modeled using an Arrhenius energy landscape (E_n, B_{ij}) and a cyclic drive $(\sum_{(ij) \in c} F_{ij})$ that tilts the landscape. The primed variables are the unprimed shifted by $\sum F_{ij}$. There is a gauge ambiguity in distributing the drive onto each edge—Many combinations of (E, B, F) lead to the same dynamics. (d) CFT resolves this ambiguity by deriving conjugate forces as affinities to node dwelling, edge exchange, and cycle completion.

$[\text{ATP}]/c^\circ$; $c_{ij} = 1$ for steps that bind nothing). Such Markov jump models are constrained by the local detailed balance (LDB) condition [28–30], relating the cycle affinity of any cycle c to the thermodynamics of the EQ reservoirs:

$$\sum_{(ij) \in c} \ln \frac{k_{ij}}{k_{ji}} = \frac{\Delta\mu - w}{k_B T}, \quad (1)$$

where $\Delta\mu$ is the free-energy consumption after completing the cycle and w is the work done by the cycle. The concentrations enter Eq. (1) through $\Delta\mu$, e.g., $\Delta\mu = -\Delta G^0 + k_B T \ln([\text{ATP}]/[\text{ADP}][P_i])$ for ATP hydrolysis. Designing a machine means changing its rate constants k_{ij}^0 while the concentrations, and hence each $\Delta\mu$, stay fixed. We describe these changes as shifts of state energies E_i and kinetic barriers B_{ij} (in $k_B T$ units). By Arrhenius kinetics, $k_{ij}^0 \propto \exp[-(B_{ij} - E_i)]$. Raising E_n multiplies every rate out of node n by the same factor. Raising B_{ij} divides k_{ij} and k_{ji} by the same factor. These two perturbation types are the ones we use throughout this paper [Eqs. (4) and (5a)].

More generally, past work parametrizes the full rates in an Arrhenius-like form [31] [Fig. 2(c)]:

$$k_{ij} = \mathcal{N} \exp[-(B_{ij} - E_i)] \exp(F_{ij}/2), \quad (2)$$

where $\mathcal{N}$ is a global timescale prefactor and the local edge affinities $F_{ij} = -F_{ji}$ distribute the cycle affinities of Eq. (1) over the edges. The purpose of this form is to classify rate perturbations into three types. Besides E and B , raising F_{ij} multiplies k_{ij} and divides k_{ji} by the same factor. Each type obeys its own response relations, which this classification

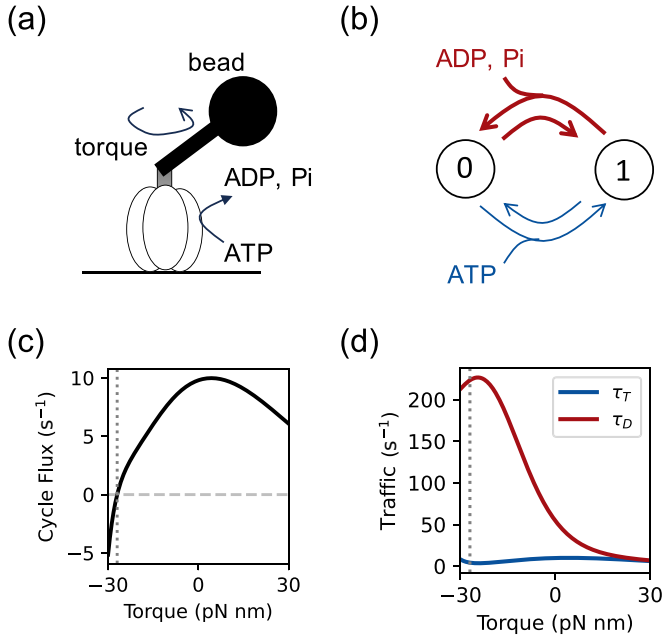


FIG. 3. The F₁-ATPase rotary motor properties. (a) In the Gerritsma and Gaspard assay, the motor is fixed to the surface and the bead rotates [19]. (b) The two-state Markov network: ATP binding (blue) and catalysis (red) transitions. Side lines mark molecules that bind to or leave the machine during a transition. A side line that merges into an arrow marks a reactant (ATP); one that branches off marks a product (ADP and P_i). Each side line serves both directions of its transition: A reactant of the forward step is a product of the reverse step. The F₁ motor rotates 120° every time a 0-1-0 (de)hydrolysis cycle is completed. (c) The steady-state cycle flux and (d) the symmetric traffics on the two edges vs the applied external torque. The traffic imbalance ($\tau_D \gg \tau_T$) indicates the motor's processive inefficiency *in vitro*. We reproduce panel (c) in Fig. 8 of Gerritsma and Gaspard [19] and use their data-fitted model to compute traffics and plot panel (d).

helped discover [31–34]. However, the parameters E , B , and F do not provide generic, one-to-one conjugate relations to the dynamical observables $\bar{\mathbf{x}} = (\boldsymbol{\pi}, \boldsymbol{\tau}, \mathbf{J})$. The parameter space (E, B, F) has more degrees of freedom than the linearly independent observables. As a result, many distinct parameter sets produce exactly the same dynamics, a gauge degeneracy (Sec. I A of the Supplemental Material [35]). A concentration is one example: Its effect on the rates can be split among E , B , and F in many ways. To get generic design principles independent of system details, resembling those that underlie the power of EQ thermodynamics, we must first identify the true conjugate forces of NEQ dynamics.

Just as EQ entropic forces (like $1/T$) are defined as the gradients of state entropy, CFT derives a set of “path-entropic forces” via the maximization of path entropy (maximum caliber) for Markov dynamics [17,38,39]. These forces are measured from a reference process with rates u_{ij} , which serves as the zero-force baseline. We set $u_{ij} = 1$ (in the time unit of k_{ij}) on every transition of the network where $k_{ij} > 0$, and $u_{ij} = 0$ elsewhere. The forces conjugate to the node distribution π_n , the edge traffic τ_{ij} , and the cycle fluxes J_c represent the entropic affinities for node dwelling, edge exchange, and

cycle completion, respectively [Fig. 2(d)]:

$$\begin{aligned}\mathfrak{F}_{\text{node},n} &= \sum_{i(\neq m)} (k_{mi} - u_{mi}) - \sum_{j(\neq n)} (k_{nj} - u_{nj}), \\ \mathfrak{F}_{\text{edge},ij} &= \frac{1}{2} \ln \frac{k_{ij}k_{ji}}{u_{ij}u_{ji}}, \\ \mathfrak{F}_{\text{cycle},c} &= \frac{1}{2} \sum_{(ij) \in c} \ln \frac{k_{ij}}{k_{ji}} - \frac{1}{2} \sum_{(ij) \in c} \ln \frac{u_{ij}}{u_{ji}}.\end{aligned}\quad (3)$$

Note that in $\mathfrak{F}_{\text{node},n}$, the index m on the right represents the arbitrarily chosen reference node when constructing the linearly independent set of distribution π_n . The reference process u only sets which dynamics has zero force. Changing u_{ij} , including a change of time unit, shifts each force by a constant. In CFT Design, we vary k_{ij} and never u_{ij} , so all response relations below are independent of this choice. For the unit-rate reference $u_{ij} = 1$ used here, the baseline constants vanish for the edge and cycle forces. For the node force, the constant is $N_n - N_m$, the difference between the out-degrees of nodes n and m . In deriving these forces, we have assumed “microinvertibility” [40], meaning that every forward transition has a corresponding reverse transition ($k_{ij} > 0 \iff k_{ji} > 0$). Cases involving “noninvertible” steps ($k_{ji} = 0$ while $k_{ij} > 0$) will lead to dropping specific edge forces (as fixing each $k_{ji} = 0$ reduces one degree of freedom) and a different form of cycle forces, as shown in later sections. CFT handles the LDB constraint naturally as $\mathfrak{F}_{\text{cycle},c}$ is half of the cycle affinity. These forces enable NEQ design in two following ways.

A. Conjugate coordinates solve constrained optimization

NEQ design first requires determining the optimal performance limits under physical constraints. CFT is effective when the performance metrics can be spanned by long-term steady-state distributions $\boldsymbol{\pi}$, traffics $\boldsymbol{\tau}$, or net fluxes $\mathbf{J}$. For instance, in our other work on adaptive dynamics [41], we demonstrated how CFT maps long-term population growth to state distributions and specific net fluxes. Here, we bring this distribution-flux anatomy to molecular machines, where performance is primarily mapped to cycle net fluxes and edge traffics. We provide examples where combinations of these observables define the processive efficiency of ATP synthase, the efficiency of enzyme inhibitors, and the speed/accuracy/cost of kinetic proofreaders.

Furthermore, designing molecular machines requires handling cycle force constraints based on LDB, such as clamping the chemical potential of energy sources (e.g., ATP, GTP) or fixing it for futile cycles. CFT solves this using its conjugate coordinate systems and Legendre transformations [17]. By shifting from a purely observable coordinate basis $(\boldsymbol{\pi}, \boldsymbol{\tau}, \mathbf{J})$ to a mixed observable-force coordinate basis such as $(\boldsymbol{\pi}, \boldsymbol{\tau}, \mathfrak{F}_{\text{cycle}})$ (see Sec. I A of the Supplemental Material [35]), we can simply hold the cycle force constraints ($\mathfrak{F}_{\text{cycle}}$) constant and tune the remaining parameters to meet functional targets and inversely solve for the necessary underlying rates k_{ij} .

This approach previously yielded the equal traffic principle (ETP) [17]—the rule that maximizing cycle flux in

a unicircular process under a fixed total traffic budget requires maintaining equal traffic across all steps. Here, we recapitulate this principle to diagnose the efficiency of the F_1 molecular motor, extend it to multicircular processes for the design of stronger enzyme inhibition, and use CFT observable-force coordinates to inversely design the global rate-driving protocols for simultaneously optimizing the speed, accuracy, and cost of kinetic proofreaders—breaking the commonly believed, inevitable trade-offs.

B. CFT forces yield flow response rules

The second task in NEQ design is to predict how tuning system parameters affects the network's long-term flow patterns. CFT establishes far-from-EQ generalizations to Maxwell-Onsager symmetries and fluctuation-response equalities, mapping how dynamic observables respond to force perturbations [17,18]. These general force-response and rate-response relations are fundamental to any ergodic Markov jump processes and do *not* require LDB connecting cycle force to energetics [Eq. (1)] or the Arrhenius parametrization to classify responses [Eq. (2)]. Because CFT forces form a complete coordinate system for the Markov dynamics, any arbitrary parameter perturbation (such as varying specific sets of the transition rates) can be mathematically expanded in terms of these force perturbations via chain rules. For example, changing a concentration rescales the rates of the steps that bind that molecule.

As set up above, a machine in a fixed environment is designed through its state energies E_n and barriers B_{ij} , its evolutionary degrees of freedom: They reshape the energy landscape, while the cycle forces stay fixed. By expanding these E and B parameter tunings in terms of the underlying force coordinates (Sec. I B of the Supplemental Material [35]), generic response relations in perturbing E_n and B_{ij} have been obtained via CFT's force response symmetries [18]. This coordinate transformation unites various recently discovered response relations [31,33,34,42] under a single principled entropic-force framework. In this paper, we turn these response relations into actionable design rules, focusing on two particularly useful response relations.

(1) *Node energies affect the scaling of fluxes.* Consistent with recent findings [31,34,42], CFT derives that node energy perturbations isotropically scale the average value of any flux variable $\bar{\varphi} \in (\tau_{ij}, J_c)$ (or their linear combinations):

$$\frac{\partial \bar{\varphi}}{\partial E_n} \Big|_{\text{Eq. (2)}} = \sum_{j(\neq n)} k_{nj} \frac{\partial \bar{\varphi}}{\partial k_{nj}} \Big|_{\text{(CFT)}} = \pi_n \bar{\varphi}, \quad (4)$$

showing that the state (node) with highest π_n is the most effective scaler. Intuitively, perturbing E_n alters how easy it is to escape state n . Increasing E_n makes all escape rates k_{nj} higher while simultaneously decreasing π_n and increasing other π . The nontrivial aspect of Eq. (4) is that E_n tuning scales *all* fluxes by the exact same proportion regardless of whether the fluxes are connected to node n . This gives two immediate implications. First, tuning node energies cannot change the accuracy of kinetic-proofreading (KP) networks, as independently shown by others [42]. Second, node energy modulates the entropy production rate (EPR) of generic networks: $\partial_{E_n} \text{EPR} \equiv \partial_{E_n} [\sum_c J_c (2\mathfrak{F}_{\text{cycle},c})] =$

$\pi_n \text{EPR}$, where we have used $2\mathfrak{F}_{\text{cycle},c} = \sum_{(ij) \in c} \ln(k_{ij}/k_{ji}) = 2 \sum_{(ij) \in c} \text{arctanh}(J_{ij}/\tau_{ij})$, confirming that its derivative with respect to E_n vanishes.

(2) *Kinetic barriers affect the routing of fluxes.* Conversely, we recover the recently reported result [33] that barrier perturbations B_{ij} route fluxes, mediated by the edge's net flux J_{ij} , and evaluate them efficiently by CFT's Jacobian matrix approach. For any distribution or flux observable $\bar{x}_\alpha \in (\pi_n, \tau_{ab}, J_c)$ (except the local traffic τ_{ij} , which has an additional term in the equation [35]),

$$\frac{\partial \bar{x}_\alpha}{\partial B_{ij}} \Big|_{\text{Eq. (2)}} = -k_{ij} \frac{\partial \bar{x}_\alpha}{\partial k_{ij}} - k_{ji} \frac{\partial \bar{x}_\alpha}{\partial k_{ji}} \quad (5a)$$

$$\stackrel{\text{(CFT)}}{=} -J_{ij} A_{\alpha,(ij)}^{-1}, \quad (5b)$$

where A^{-1} is the inverse of the force-rate Jacobian matrix $A_{(ij),\alpha} \equiv k_{ij} \partial_{k_{ij}} \mathfrak{F}_\alpha$, easily evaluated based on Eq. (3) [18,35]. Intuitively, tuning a kinetic barrier modulates local kinetic resistance. When a barrier is raised, it redistributes traffic within the same cycle and redirects flux into alternative pathways branching from the same node. The quantitatively nontrivial aspect of Eq. (5a) is that tuning a barrier that carries no net flux alters only its own traffic τ_{ij} while leaving *all* other network observables invariant. We will show how this topological routing rule establishes the design limits of enzyme inhibitors.

These relationships constitute a dual design framework for Markov processes and the molecular machines they model: inverse design (solving constrained optimal rate configurations via conjugate coordinates) and forward design (routing flows via fluctuation-response rules). The two tools differ in reach. Forward design is local: The response rules give gradients, which tell the direction in which to tune a parameter. Inverse design is not limited to small steps. Since the coordinates map one to one to the rates, any target point, including a constrained optimum, gives a unique rate configuration. Such an optimum can lie inside the feasible domain or on its boundary. It lies on the boundary when the performance metric is itself a coordinate, because an interior point can always move further along that coordinate. The examples below show both cases. We demonstrate the framework with three examples:

(1) *Motor efficiency.* A data-fitted, unicircular F_1 motor serves as the simplest, illustrative example.

(2) *Kinetic proofreading.* Our main example showing how CFT reveals ways to overcome the trade-offs among speed, accuracy, and costs in data-fitted proofreading model of *E. coli* ribosome.

(3) *Enzyme inhibition.* A demonstration of how CFT generalizes to microscopically noninvertible models (some $k_{ji} = 0$), revealing the different design rules for enzyme inhibitors with different model topologies.

We present the recipe of this framework in Sec. I C of the Supplemental Material [35].

III. EXAMPLE 1: SPEEDING UP MOTORS THROUGH TRAFFIC CONTROL

The F_0 - F_1 ATP synthase is biology's rotary transducer: The F_0 motor converts the downhill flow of protons into

mechanical torque, which drives the F_1 motor to perform the energetically uphill synthesis of ATP. While earlier models evaluate its *thermodynamic efficiency* (work output versus free energy consumed) [1,2], we show here that its speed and utility are also constrained by its *processive efficiency*, defined as the ratio of net transport to total transitions taken (J/τ). Here, we show that the F_1 motor is limited by futile backstepping, a traffic imbalance that can only be corrected by routing flows via kinetic barriers.

To model the *in vitro* F_1 motor assay [Fig. 3(a)], we adopt the two-state, two-edge, multigraph network from Gerritsma and Gaspard [19] [Fig. 3(b)]. Under the low-ATP conditions in the experiments ($\approx 0.5 \mu\text{M}$), the slow ATP-binding rate justifies lumping the subsequent transitions in catalysis and product release. The motor transitions between an empty (node 0) and a nucleotide-occupied state (node 1) via the T transition (ATP binding/unbinding, $\approx 90^\circ$ rotation) and the D transition (catalysis and product release, $\approx 30^\circ$ rotation). The reverse of D lumps the rebinding of ADP and P_i into one trimolecular step, with a rate proportional to $[\text{ADP}][P_i]$ (Sec. II A of the Supplemental Material [35]). The model fits well to the experimental data [19].

The network dynamics are governed by four torque-dependent transition rates, $k_{T\pm}(\Gamma)$ and $k_{D\pm}(\Gamma)$, where Γ is the applied torque (see Sec. II A of the Supplemental Material [35]). Following Ref. [19], we confine our analysis to $\Gamma \in [-30, 30] \text{ pN nm}$ to preclude mechanical slipping and maintain the tight-coupling condition. These phenomenological rates satisfy the LDB cycle force constraint:

$$2\mathfrak{F}_{\text{cycle}} \stackrel{(\text{CFT})}{=} \ln \frac{k_{T+}(\Gamma)k_{D+}(\Gamma)}{k_{T-}(\Gamma)k_{D-}(\Gamma)} \stackrel{(\text{LDB})}{=} \frac{\Delta\mu + \Gamma\Delta\theta}{k_B T}, \quad (6)$$

where $\Delta\theta = 2\pi/3$ is the rotation angle and $\Delta\mu$ is the chemical free energy of ATP hydrolysis. Of interest are the steady-state distribution of the two states (π_0, π_1), the edge traffics (τ_T, τ_D), and the cycle flux (J_c), representing 1/3 of the motor's physical rotation speed.

CFT's observable-force coordinate ($\pi_1, \tau_T, \tau_D, \mathfrak{F}_{\text{cycle}}$) diagnoses the motor's processive inefficiencies by decoupling the constrained free-energy drive ($\mathfrak{F}_{\text{cycle}}$) from the tunable kinetic variables. Under a fixed total kinetic activity budget ($\tau_{\text{tot}} = \tau_T + \tau_D$), maximizing the cycle flux J_c requires the traffic to be balanced across all steps ($\tau_T = \tau_D$) [17]. This *equal traffic principle* bounds the motor's processive efficiency (J_c/τ_{tot}) (see Sec. II B of the Supplemental Material [35]):

$$\frac{J_c}{\tau_{\text{tot}}} \leq \frac{1}{2} \tanh\left(\frac{\mathfrak{F}_{\text{cycle}}}{2}\right). \quad (7)$$

The processive efficiency J_c/τ_{tot} is not itself a coordinate, and this optimum ($\tau_T = \tau_D$) lies inside the feasible domain. As shown in Fig. 3(d), the *in vitro* rotor operates far below this optimum. The traffic imbalance ($\tau_D \gg \tau_T$) indicates that kinetic activity is wasted on futile backstepping along the D transition.

To see how one could correct this imbalance under a fixed thermodynamic drive ($\mathfrak{F}_{\text{cycle}}$), we compute all energetic response relations of this network [Eqs. (4) and (5a)] from a

single analytical Jacobian inverse (see Secs. II C and II D of the Supplemental Material [35]):

$$\frac{\partial \ln J_c}{\partial E_n} = \frac{\partial \ln \tau_T}{\partial E_n} = \frac{\partial \ln \tau_D}{\partial E_n} = \pi_n, \quad (8a)$$

$$\frac{\partial \ln J_c}{\partial B_T} = -\frac{\Sigma_D}{\Sigma}, \quad \frac{\partial \ln J_c}{\partial B_D} = -\frac{\Sigma_T}{\Sigma}, \quad (8b)$$

$$\frac{\partial \ln \tau_T}{\partial B_T} = \frac{J_c}{\tau_T} \frac{\Delta_T}{\Sigma} - 1, \quad \frac{\partial \ln \tau_T}{\partial B_D} = -\frac{J_c}{\tau_T} \frac{\Delta_T}{\Sigma}, \quad (8c)$$

$$\frac{\partial \ln \tau_D}{\partial B_D} = \frac{J_c}{\tau_D} \frac{\Delta_D}{\Sigma} - 1, \quad \frac{\partial \ln \tau_D}{\partial B_T} = -\frac{J_c}{\tau_D} \frac{\Delta_D}{\Sigma}. \quad (8d)$$

Here, $\Sigma_X = k_{X+} + k_{X-}$, $\Delta_X = k_{X+} - k_{X-}$, and Σ is the sum of all rates. These relations prescribe how to systematically design the motor to reach its maximum processive efficiency.

First, Eq. (8a) verifies that perturbing the node energy E_n scales all fluxes equally. Hence, tuning node energies cannot correct the motor's traffic imbalance ($\partial_{E_n} \ln[\tau_T/\tau_D] = \pi_n - \pi_n = 0$).

Therefore, improving efficiency requires tuning the kinetic barriers to route the internal traffic. We can verify this by directly subtracting Eq. (8c) from Eq. (8d):

$$\frac{\partial \ln(\tau_T/\tau_D)}{\partial B_T} = -\frac{\partial \ln(\tau_T/\tau_D)}{\partial B_D}, \quad (9)$$

showing that the traffic imbalance is fixable by the kinetic barriers. Selectively raising the catalysis barrier (B_D) or lowering the binding barrier (B_T) can balance traffic distributions and thus improve its processive efficiency J_c/τ_{tot} for a given chemical drive.

Finally, the fact that processive efficiency is completely insensitive to E_n highlights a structural degeneracy inherently mapped by the CFT coordinate ($\pi_1, \tau_T, \tau_D, \mathfrak{F}_{\text{cycle}}$). Because the efficiency bounds only depend on the traffic variables and $\mathfrak{F}_{\text{cycle}}$, multiple different rate configurations can achieve the exact same efficiency [17]. It was reported that this efficiency degeneracy can be navigated by changing all rates according to $\mathbf{k}(\pi_1, \tau_T, \tau_D, \mathfrak{F}_{\text{cycle}})$, with varying π_1 and other parameters fixed. Here, Eq. (8a) shows that tuning E_n gives a localized protocol to navigate this degenerate design space, allowing one to adjust state occupancies (π_n) without disrupting the optimal traffic balance by tuning only local energy levels E_n .

In summary, the *in vitro* F_1 -ATPase operates far from its optimal processive efficiency, due to considerable backstepping. CFT gives design insights. Processive efficiency is about the directionality of fluxes, not about how the system is distributed over nodes in time (π_n) or how easy it is to escape each node (E_n). While node energies scale the motor activity within a degenerate space of state occupancies, the kinetic barriers affect the routing and can correct this traffic imbalance.

IV. EXAMPLE 2: DECOUPLING SPEED, ACCURACY, AND COST IN KINETIC PROOFREADING

Ninio [20] and Hopfield [21] were the first to explain how biomolecular processes can harness NEQ driving to achieve certain biochemical process accuracies beyond the

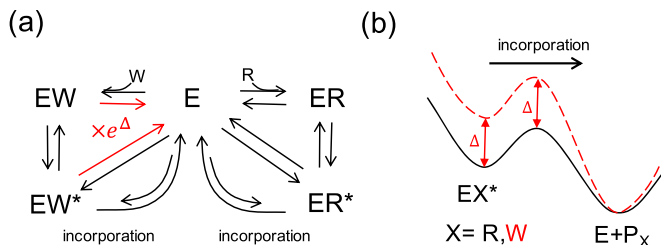


FIG. 4. The classical model of kinetic proofreading assumes barrier asymmetry. (a) Traditional models assume that discriminating right (R) from wrong (W) relies solely on accelerating the unbinding of the W substrate, while keeping forward incorporation rates identical. (b) Because the wrong bound state (red dashed) is destabilized by a node energy Δ , maintaining identical incorporation rates mathematically requires elevating the wrong kinetic barrier by exactly the same Δ . Side lines (P_R, P_W) follow the convention of Fig. 3(b).

limits of thermodynamic EQ. For example, in *kinetic proofreading*, a ribosome translates mRNA with an error rate of approximately one in 500 amino acids, and a DNA polymerase enzyme can copy DNA sequences onto new DNA with extremely small error rates, of one in 10^9 monomers. Traditionally, KP is viewed as trade-offs among speed, accuracy, and energy cost [43,44]. However, our design framework reveals that these canonical trade-offs are not absolute; they only manifest at the boundaries of the system's design space. Within the operational interior, these performance metrics can be decoupled. We show here how a specific kinetic barrier can act as a topological router to break the trade-offs, and remarkably, how real proofreading networks seem to have evolved quite close to the optimal saturation point.

While implicit, the necessity of barrier routing is embedded in foundational KP models [20,21]. Classic theories attributed error correction primarily to differing unbinding rates for the right (cognate) and wrong (noncognate) substrates, while assuming identical forward incorporation rates [black curved arrows in Fig. 4(a)]. CFT Design reveals a hidden topological constraint. As established earlier, node energies (E_n) only scale fluxes ($\partial \ln J_c / \partial E_n = \pi_n$). Thus, node energy differences alone cannot alter the error rate [$\varepsilon = \log_{10}(J_W/J_R)$], as independently reported by others [42]. To maintain identical incorporation rates while altering unbinding rates, the classic model implicitly assumes asymmetric incorporation barriers across the right or wrong substrates [Fig. 4(b)]. Barrier routing, not just binding affinity, is essential to proofreading.

To move beyond idealized symmetric models, we apply our framework to the asymmetric networks of real KP systems mapped by recent single-molecule experiments [22,23]. We focus on the Markov model for *E. coli* ribosomes here. The model has five states [Fig. 4(a)]. In state E, the ribosome waits for the next aminoacyl-tRNA. Each substrate, R or W, is a tRNA that carries the right or wrong amino acid and brings one GTP molecule with it. The substrate binds to the codon, giving state ER or EW. Its GTP is then hydrolyzed, giving the activated state ER* or EW*. From an activated state, the ribosome returns to E by one of two steps. Either the amino acid is added to the growing protein (incorporation) or the tRNA is rejected (proofreading discard).

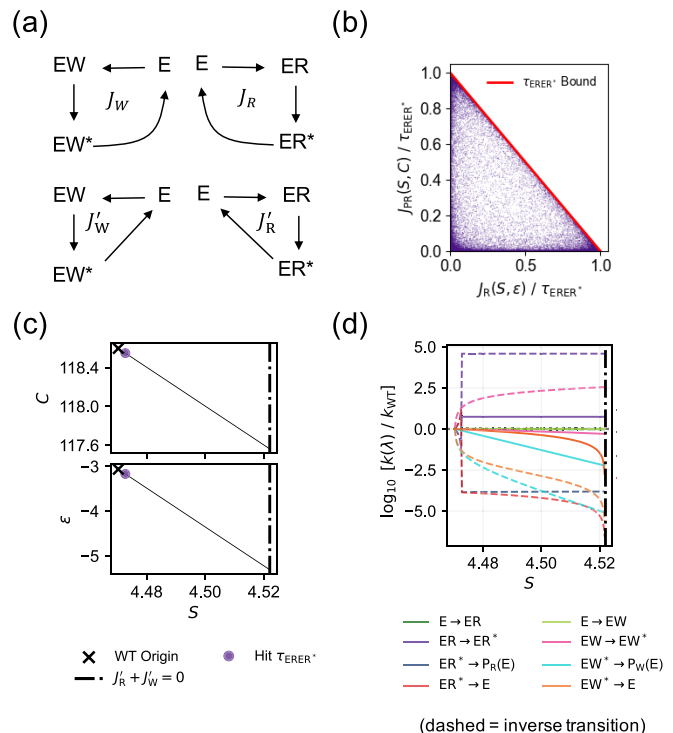


FIG. 5. Inverse design of the *E. coli* ribosome. (a) The four fundamental cycles defining the incorporation (J_R, J_W) and proofreading (J'_R, J'_W) fluxes. (b) Dots sample 10^5 transition rate configurations with values log-uniformly distributed from 10^{-4} to 10^4 . Four specific rates are not sampled but computed from other sampled rates to meet the cycle force constraints for the four hydrolysis cycles. Bound saturates when $J_R + J'_R = \tau_{ER,ER^*} \Leftrightarrow P_{ER,ER^*} = 0$. (c) An inversely designed “evolutionary” process increasing speed S while reducing error ε and cost C from the wild-type origin (black cross). Purple dots indicate where specific traffic limits ($\sum J \leq \tau$) saturate and are enlarged tenfold to allow further optimization. The vertical dash-dotted line marks the zero-proofreading boundary ($J'_W + J'_R = 0$). (d) Solving the driving protocol of transition rates realizing the linear inverse design path in panel (c). The jumps in rates near onset are caused by the traffic limit relaxation.

A. Trade-offs emerge only at the parameter-space boundaries

We use CFT's coordinate systems to show that speed-accuracy-cost trade-offs are boundary constraints. To demonstrate this in the *E. coli* ribosome, we formalize the network's performance using four fundamental cycle fluxes [Fig. 5(a)]: correct and incorrect incorporations (J_R, J_W) and their corresponding proofreading discards (J'_R, J'_W). The performance metrics are algebraic combinations of these cycle fluxes: total incorporation speed $S = J_R + J_W$, error $\varepsilon = \log_{10}(J_W/J_R)$, and power cost $C = S \Delta\mu_p + (J'_R + J'_W) \Delta\mu_{GTP}$, where $\Delta\mu_p$ and $\Delta\mu_{GTP}$ are the free energies of peptide bond formation and GTP hydrolysis, fixed throughout this example.

We use CFT's force-observable coordinate $\mathbf{z} = (\pi, \tau, \mathcal{F}_{\text{cycle}})$ to map these performance targets back to the underlying transition rates. It takes three steps. (1) We choose the spanning tree $E \rightleftharpoons ER \rightleftharpoons ER^*$ and $E \rightleftharpoons EW \rightleftharpoons EW^*$. The four remaining edges, or “chords,” are the incorporation and discard steps $ER^* \rightarrow E$ and $EW^* \rightarrow E$. Each chord closes one fundamental cycle [Fig. 5(a)]. (2) On each chord

(ab), the cycle force fixes the chord traffic:

$$\tau_c = J_c \coth \left[\mathfrak{F}_{\text{cycle},c} - \sum_{(ij) \in c \setminus (ab)} \operatorname{arctanh}(J_{ij}/\tau_{ij}) \right]. \quad (10)$$

The net flux J_{ij} on each tree edge is a sum of cycle fluxes, so at fixed $\mathfrak{F}_{\text{cycle}}$, the four cycle fluxes can replace the four chord traffics. The traffics on the tree edges remain as the vector τ_{rem} . (3) The four cycle fluxes and the four targets (S, ε, C, r') determine each other one to one:

$$J_R = \frac{S}{1 + 10^\varepsilon}, \quad J_W = 10^\varepsilon J_R, \quad (11a)$$

$$J'_R = \frac{C - S\Delta\mu_p}{\Delta\mu_{\text{GTP}}(1 + r')}, \quad J'_W = r'J'_R, \quad (11b)$$

where $r' = J'_W/J'_R$ is the discard ratio. So, S, ε, C , and r' can serve as independent variables in place of the four cycle fluxes. This gives the functional coordinate $\zeta = (\pi, \tau_{\text{rem}}, S, \varepsilon, C, r', \mathfrak{F}_{\text{cycle}})$. This yields a parametrization $k_{ij}(\zeta)$ that translates target performances and cycle force constraints directly into the required transition rate configurations. See Sec. III A of the Supplemental Material [35] for more details of its construction.

This yields performance bounds dictated by the non-negativity of one-way transition fluxes ($p_{ij} = [\tau_{ij} + J_{ij}]/2 \geq 0$). For example, the kinetic capacity of the hydrolysis step of the right substrate imposes the constraint $J_R(S, \varepsilon) + J'_R(S, C) \leq \tau_{\text{ER},\text{ER}^*}$ (i.e., $p_{\text{ER},\text{ER}^*} \geq 0$). By algebraically substituting the definitions of S, ε , and C into this capacity constraint, we obtain the following performance bound:

$$\frac{S}{1 + 10^\varepsilon} + \frac{C - S\Delta\mu_p}{\Delta\mu_{\text{GTP}}(1 + r')} \leq \tau_{\text{ER},\text{ER}^*}. \quad (12)$$

This bound [numerically verified in Fig. 5(b)] describes a traffic jam effect: Productive incorporation (the first term) and futile proofreading (the second term) must compete for a finite kinetic bandwidth ($\tau_{\text{ER},\text{ER}^*}$).

Because S, ε , and C serve as independent variables in our functional coordinate ζ , trade-offs manifest *only* at the boundary where this inequality saturates, and the optimum of each metric lies on this boundary: At any interior point, the metric can still improve along its own coordinate. As long as neither the traffic bandwidth nor other physical bounds are saturated (the feasible interior), these metrics are decoupled. This predicts that an enzyme can simultaneously improve speed, reduce error, and lower cost under fixed thermodynamic cycle drives ($\mathfrak{F}_{\text{cycle}}$). Figure 5(c) verifies this by designing a linear trajectory that optimizes all three metrics from their wild-type (WT) origins. And, by feeding this targeted linear performance path back into the CFT parametrization $k_{ij}(\zeta)$, we can calculate the global control protocol of transition rates required to realize it [Fig. 5(d)]. This shows that speed, error, and cost of kinetic proofreaders can be separately controlled under fixed cycle forces $\mathfrak{F}_{\text{cycle}}$.

The functional coordinate ζ from CFT demonstrates that, for a given linearly evolving performance requirement (S, ε, C) and fixed cycle forces $\mathfrak{F}_{\text{cycle}}$, the remaining tunable degrees of freedom are $(\pi, \tau_{\text{rem}}, r')$. By algorithmically tuning these variables, we can elongate the linear optimization

of kinetic proofreaders. We highlight two examples here using targeted variations of τ_{rem} while keeping (π, r') fixed. See Sec. III C of the Supplemental Material for algorithmic details [35].

(1) We start by fixing all τ_{rem} and varying only (S, ε, C) from the wild-type parameters [marked with x in Fig. 5(c)]. Whenever this designed optimization path saturates a local traffic bound [e.g., $p_{\text{ER},\text{ER}^*} \rightarrow 0$ in Eq. (12), marked with purple dots], we relax that limit by enlarging that specific traffic ($\tau_{\text{ER},\text{ER}^*}$) tenfold, allowing the optimization to continue. This designed, sudden expansion of traffic bandwidth dictates the necessary discrete jumps in the inversely solved transition rate $k_{ij}(\zeta)$, as shown in Fig. 5(d).

(2) This linear optimization continues until reaching the zero-proofreading flux boundary [$J'_R + J'_W = 0$, dash-dotted lines in Figs. 5(c) and 5(d)]. Crossing this boundary enters an “antiproofreading” regime ($J'_R + J'_W < 0$) [45] where the system converts the GTP-consuming proofreading cycles into GTP-synthesizing ones. This is necessary to push the enzyme to achieve even lower energy costs at higher speeds. Reaching this regime requires $\Delta\mu_p > \Delta\mu_{\text{GTP}}$ alongside targeted control of τ_{rem} . An example is shown in Sec. III C 2 of the Supplemental Material [35].

The CFT inverse design framework shows that, as long as the system remains within the feasible interior, speed, error, and cost can be optimized independently under fixed cycle forces, along any path in the interior; the linear path above only simplifies the illustration. While the optimization path we prescribe requires a “global control” with all network transition rates tuned simultaneously in specific ways, which can be demanding for practical or experimental implementation, it establishes a theoretical proof of overcoming the trade-offs. Building on this, we next introduce a more accessible, localized approach to break the trade-offs.

B. Evolutionary saturation of a critical discard barrier decouples performance trade-offs

Using our analytical barrier routing rules [Eq. (5a)], we map the performance gradients for all barrier perturbations in the *E. coli* ribosome from a single Jacobian matrix inverse [Fig. 6(a)]. This sensitivity map reveals four distinct tuning behaviors, identifying the key to achieving high speed, low error, and low cost. We show details of how the four cycle fluxes change under different barrier perturbations in Sec. III D of the Supplemental Material [35]. Here is the summary:

(1) Accelerating by lowering the noncognate (W) incorporation barrier (gray dashed line) trivially increases speed but compounds both error and cost—the worst intervention that one should avoid.

(2) Lowering barriers along the correct pathways (red dashed lines)—such as R incorporation or W proofreading—increases speed and reduces error. However, this raises the total energetic cost because it scales up the total processing flux without restricting futile energy consumption. Trade-offs persist.

(3) Raising barriers to block the W pathways (red solid lines) accelerates processing while lowering the error rate. Yet, blocking W redirects the enzyme to increase the overall

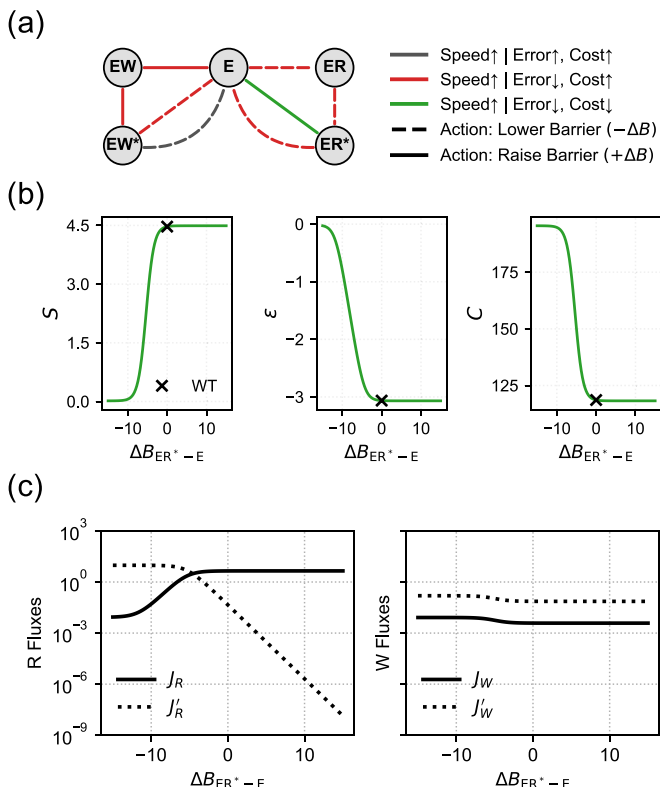


FIG. 6. Performance routing and evolutionary saturation in kinetic proofreading. (a) Barrier sensitivity maps for the *E. coli* ribosome. Colors indicate the different types of responses for each barrier perturbation. Solid and dashed lines denote raising (+ΔB) and lowering (-ΔB) barriers to increase speed, respectively. (b) Performance metrics—speed S , error ε , cost C —along the “free lunch” barrier tuning [green path in panel (a)]. The WT parameters (marked as x) reside near the saturating plateau for all three metrics. (c) Cycle fluxes along the barrier tuning. Solid and dotted lines represent product formation (J) and discard (J') fluxes, respectively, for the right (left) and wrong (right) substrates.

cycling of R, elevating both the product flux J_R and the futile discard flux J'_R . Thus, the total energy cost still increases. Trade-offs persist.

(4) Sustaining a high barrier to discarding R (B_{ER^*-E} , green line) is a critical mechanism to break trade-offs. Lowering it from the WT state facilitates the discard of R, redirecting flux away from product formation (J_R) toward futile discard (J'_R). As shown in Fig. 6(b), this causes a simultaneous drop in speed, a spike in error, and a rise in cost. On the contrary, raising the barrier simultaneously optimizes the three metrics.

Therefore, the WT system breaks the trade-off by maintaining a high discard barrier specifically for R. Raising this barrier further suppresses J'_R without limiting J_R , providing marginal gains in speed while continuing to lower both error and cost, as shown in Fig. 6(b). This simultaneous optimization without standard trade-offs constitutes a unique “free lunch.” Our results indicate that biological evolution seems to have targeted this specific topological router, pushing the barrier to a near-saturation point to optimize performance.

In summary, while the canonical understanding of speed-accuracy-cost trade-offs in kinetic proofreading is defined by absolute boundary constraints [43,44,46,47], these performance metrics can be decoupled within the operational interior. This aligns with the emerging realization that NEQ dynamics can circumvent static trade-offs by exploiting localized kinetic degrees of freedom [48]. Evolution appears to have navigated this interior region by pushing the cognate discard barrier along a “free lunch” tuning direction toward near-saturation.

V. EXAMPLE 3: DESIGNING MORE EFFICIENT ENZYME INHIBITORS

Classical pharmacology evaluates enzyme inhibitor efficacy based on binding affinities, $\ln(k_{on}/k_{off})$, of the inhibitor molecule to the target enzyme [25]. By treating Michaelis-Menten (MM) inhibition as an NEQ flow-routing problem, CFT Design shows that inhibitor efficacy fundamentally depends on network topology.

To analyze these networks, we must first address the catalytic step ($ES \rightarrow E + P$), which is commonly modeled as noninvertible. This noninvertibility is a standard and convenient approximation representing product release under dilute physiological conditions or fast product removal, where the probability of reverse binding is negligible ($k_{cat-reverse} \rightarrow 0$). We extend CFT—originally formulated for invertible networks where all $k_{ji} > 0$ —to this zero-rate limit. Because the catalytic transition is noninvertible, its symmetric traffic equals its directed flux ($\tau_{cat} = J_{cat}$) and is thus not needed in the distribution-flux coordinate $\mathbf{x} = (\boldsymbol{\pi}, \boldsymbol{\tau}, \mathbf{J})$. By selecting a spanning tree where this noninvertible edge acts as a fundamental cycle chord, we construct the updated $\mathbf{x}$ without τ_{cat} and plug that into maximum caliber. It reveals that most conjugate forces preserve their original forms (see Sec. IV A of the Supplemental Material [35]). The single modification occurs on the catalytic cycle: The absence of a reverse transition alters its force contribution from $\frac{1}{2}[\ln(k_+/k_-) - \ln(u_+/u_-)]$ to $\ln(k_{cat}/u_{cat})$, yielding the modified cycle force:

$$\tilde{\mathfrak{F}}_{cat} = \ln(k_{cat}/u_{cat}) + \frac{1}{2} \left[\ln \left(\frac{k_{E,ES}}{k_{ES,E}} \right) - \ln \left(\frac{u_{E,ES}}{u_{ES,E}} \right) \right]. \quad (13)$$

As before, we set all $u_{ij} = 1$. Another choice would shift $\tilde{\mathfrak{F}}_{cat}$ only by a constant, so the design analysis is unaffected. With this observable-force conjugacy established, CFT’s analytical machinery remains fully valid for MM networks. We now use it to evaluate classical inhibition archetypes.

We first demonstrate that competitive and uncompetitive inhibitors act as topological dead ends optimized solely by node energies (Fig. 7). The inhibitor-bound states (EI and EIS) form dead-end pathways that carry zero net flux ($J_{sideway} = 0$). According to CFT’s barrier routing rule [Eq. (5a)], because $J_{ij} = 0$ along these edges, the sensitivity of any distribution or flux observable on the main pathway vanishes identically ($\partial \bar{x}_\alpha / \partial B_{dead\ end} = 0$). Consequently, tuning the kinetic barriers along dead-end pathways has no effect on catalytic production. To optimize dead-end inhibitors, rational design cannot rely on barrier routing; it must instead deepen the node energy

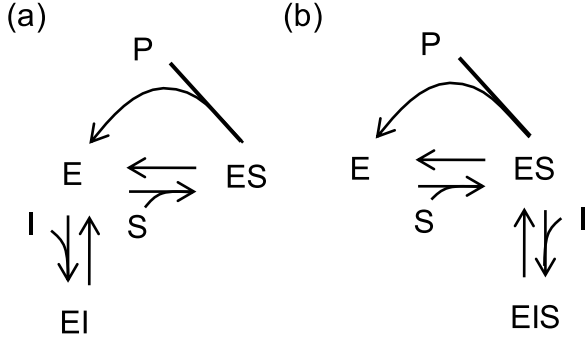


FIG. 7. Competitive and uncompetitive inhibitors rely on topological dead ends. Network diagrams for (a) competitive and (b) uncompetitive inhibition. Unlike the noncompetitive loop, the inhibitor-bound states (EI and EIS) form dead-end pathways carrying zero steady-state net flux. This topological distinction renders their long-term catalytic rates completely insensitive to kinetic barrier perturbations along the inhibitor pathways. Side lines follow the convention of Fig. 3(b); the single curved arrow marks the noninvertible catalytic step that releases P.

(E_n) of the bound state, maximizing binding affinity purely by suppressing k_{off} .

In contrast, noncompetitive inhibitors with leaky loops require kinetic barrier routing for optimization. These inhibitors form a closed loop ($E \rightleftharpoons EI \rightleftharpoons EIS \rightleftharpoons ES \rightleftharpoons E$) that couples to the catalytic step [Fig. 8(a)]. Although this internal futile cycle has zero cycle force ($\mathfrak{F}_{\text{leak}} = 0$), the noninvertible catalysis continually depletes ES and replenishes E. This tilted distribution gradient acts as a pump, driving a compensatory clockwise leak flux ($J_{\text{leak}} > 0$) through the indirect inhibitor pathway (see Sec. IV B of the Supplemental Material for proof [35]). Because of this nonzero leak flux, kinetic barriers act as effective control knobs for noncompetitive inhibition.

To optimize this network, we derive the *inhibitor equal traffic principle* as a multicircular generalization of the ETP reported in Ref. [17]. Using CFT to construct a functional coordinate $\mathbf{z} = (\boldsymbol{\pi}, \boldsymbol{\tau}, J_{\text{leak}}, \mathfrak{F}_{\text{leak}})$, we express the catalytic production [35]:

$$J_{\text{cat}} = J_{\text{leak}} + \tau_{E,ES} \tanh(\mathcal{A}_{\text{side}}), \quad (14)$$

where $\mathcal{A}_{\text{side}} = \sum_{\eta} \text{arctanh}(J_{\text{leak}}/\tau_{\eta})$ is the total affinity across the three sideways edges of the loop, indexed by $\eta \in \{E\text{-EI}, \text{EI-EIS}, \text{EIS-ES}\}$. Minimizing J_{cat} under a fixed total traffic budget requires minimizing $\mathcal{A}_{\text{side}}$, which dictates equalizing traffic across these sideways edges. This bounds the dynamical efficiency of *enzyme inhibition*: Maximizing the suppression of catalytic throughput (J_{cat}) per kinetic activity budget requires a balanced traffic distribution across the leak loop. And, just as the ETP case in Example 1, the equal traffic distribution design requires barrier tuning—node energies as flux scalars will not help. Conversely, maximum catalytic production occurs at the traffic upper bound ($J_{\text{cat}}^{\text{max}} = J_{\text{leak}} + \tau_{E,ES}$), where the reverse one-way flux $p_{ES,E}$ vanishes. This example thus shows both cases: The lower bound (equal traffic) lies inside the feasible domain, and the upper bound lies on its boundary. We numerically validate these bounds in Fig. 8(c).

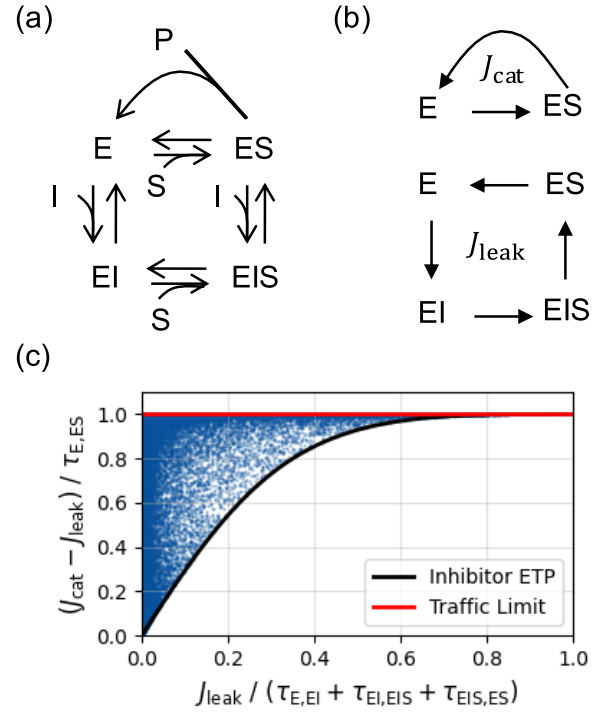


FIG. 8. Inhibitor equal traffic principle in noncompetitive enzyme inhibition. (a) The four-state network with a noninvertible catalytic step ($ES \rightarrow E + P$). Side lines follow the convention of Fig. 3(b). (b) The two fundamental cycle fluxes: the noninvertible catalytic flux (J_{cat}) and the invertible leak flux (J_{leak}). (c) Numerical validation of the operational bounds. The blue scatter points represent 10^5 randomly sampled kinetic networks with log-uniform transition rates spanning 6 orders of magnitude, constrained to maintain a futile inhibitor loop ($\mathfrak{F}_{\text{leak}} = 0$). For a given J_{leak} , catalytic production is minimized when kinetic traffic is equally distributed across the sideways edges of the inhibitor loop (lower bound). Production is maximized when the reverse one-way flux to the free enzyme vanishes ($p_{ES,E} \rightarrow 0$, upper bound).

In summary, extending CFT to catalytic networks recasts enzyme inhibition from an EQ affinity problem into an NEQ topological flow-routing problem. Competitive and uncompetitive inhibitors act as topological dead ends, completely blind to barrier routing and reliant entirely on node energy optimization. In contrast, noncompetitive inhibitors form leaky loops, achieving maximum inhibition efficiency only when kinetic barriers balance their internal traffic. This establishes an NEQ physical basis for targeted drug design.

VI. DISCUSSION

This work reframes the design of nonequilibrium molecular machines as a flow-routing problem. Traditional thermodynamic models focus on energetic drives and correlating machine performance with overall entropy production. However, global time irreversibility metrics lack the fine-grained resolution needed to locate and resolve the *topological misflows* that degrade machine performance—such as futile backstepping in rotary motors, branched misrouting in

kinetic proofreading, or leaky bypasses in enzyme inhibition. CFT Design provides this missing resolution by pairing dynamic observables with conjugate path-entropic forces. This yields a dual design tool kit: inverse design to map target distributions and fluxes to optimal rate configurations under constraints, and forward design to distinguish whether flows should be scaled via node energies or routed via kinetic barriers.

While our examples focus on biochemical networks governed by local detailed balance and Arrhenius energetics, the core design framework is independent of these specific physical assumptions. Inherited from its path-entropy-based construction [17], CFT Design applies to any ergodic Markov jump process and can be generalized to continuous Langevin processes in principle. This implies applications to broader classes of nonequilibrium systems, ranging from ecological population dynamics [41] to macroscopic transportation networks [17]. Just as equilibrium thermodynamics provides the foundational conjugate forces for controlling static distributions, CFT Design provides them for optimizing and controlling dynamical flows.

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Y.-J.Y. and K.A.D. conceptualized the project together. Y.-J.Y. developed the framework and the results. K.A.D. supervised all developments. Both authors contributed equally to writing and editing the manuscript.

DATA AVAILABILITY

The data that support the findings of this article are openly available [52].

- [1] J. A. Wagoner and K. A. Dill, Mechanisms for achieving high speed and efficiency in biomolecular machines, *Proc. Natl. Acad. Sci. USA* **116**, 5902 (2019).
- [2] A. I. Brown and D. A. Sivak, Theory of nonequilibrium free energy transduction by molecular machines, *Chem. Rev.* **120**, 434 (2020).
- [3] M. L. Mugnai, C. Hyeon, M. Hinczewski, and D. Thirumalai, Theoretical perspectives on biological machines, *Rev. Mod. Phys.* **92**, 025001 (2020).
- [4] C. Gardiner, *Stochastic Methods: A Handbook for the Natural and Social Sciences, Springer Series in Synergetics*, 4th ed. (Springer, Berlin, 2009).
- [5] H. Qian, Phosphorylation energy hypothesis: Open chemical systems and their biological functions, *Annu. Rev. Phys. Chem.* **58**, 113 (2007).
- [6] J. M. Horowitz and M. Esposito, Thermodynamics with continuous information flow, *Phys. Rev. X* **4**, 031015 (2014).
- [7] J. M. R. Parrondo, J. M. Horowitz, and T. Sagawa, Thermodynamics of information, *Nat. Phys.* **11**, 131 (2015).
- [8] U. Seifert, From stochastic thermodynamics to thermodynamic inference, *Annu. Rev. Condens. Matter Phys.* **10**, 171 (2019).
- [9] L. Peliti and S. Pigolotti, *Stochastic Thermodynamics: An Introduction* (Princeton University Press, Princeton, 2021).
- [10] Y. Cao and S. Liang, Stochastic thermodynamics for biological functions, *Quant. Biol.* **13**, e75 (2025).
- [11] M. P. Leighton and D. A. Sivak, Flow of energy and information in molecular machines, *Annu. Rev. Phys. Chem.* **76**, 379 (2025).
- [12] C. Floyd, A. R. Dinner, A. Murugan, and S. Vaikuntanathan, Limits on the computational expressivity of non-equilibrium biophysical processes, *Nat. Commun.* **16**, 7184 (2025).
- [13] Y. Tu, Nonequilibrium thermodynamics of biochemical networks: Energetics of cellular functions, *Annu. Rev. Biophys.* **55**, 225 (2026).
- [14] H. Qian and D. A. Beard, Metabolic futile cycles and their functions: A systems analysis of energy and control, *Syst. Biol. (Stevenage)* **153**, 192 (2006).
- [15] M. Baiesi and C. Maes, Life efficiency does not always increase with the dissipation rate, *J. Phys. Commun.* **2**, 045017 (2018).
- [16] C. Maes, Frenesy: Time-symmetric dynamical activity in nonequilibria, *Phys. Rep.* **850**, 1 (2020).
- [17] Y.-J. Yang and K. A. Dill, A principled basis for nonequilibrium network flows, *Nat. Commun.* **17**, 4980 (2026).
- [18] Y.-J. Yang and K. A. Dill, Fluctuation-response design rules for nonequilibrium flows, *Phys. Rev. Res.* **8**, 043039 (2026).
- [19] E. Gerritsma and P. Gaspard, Chemomechanical coupling and stochastic thermodynamics of the F_1 -ATPase molecular motor with an applied external torque, *Biophys. Rev. Lett.* **05**, 163 (2010).
- [20] J. Ninio, Kinetic amplification of enzyme discrimination, *Biochimie* **57**, 587 (1975).
- [21] J. J. Hopfield, Kinetic proofreading: A new mechanism for reducing errors in biosynthetic processes requiring high specificity, *Proc. Natl. Acad. Sci. USA* **71**, 4135 (1974).
- [22] K. Banerjee, A. B. Kolomeisky, and O. A. Igoshin, Elucidating interplay of speed and accuracy in biological error correction, *Proc. Natl. Acad. Sci. USA* **114**, 5183 (2017).
- [23] J. D. Mallory, A. B. Kolomeisky, and O. A. Igoshin, Trade-offs between error, speed, noise, and energy dissipation in biological processes with proofreading, *J. Phys. Chem. B* **123**, 4718 (2019).
- [24] W. W. Cleland, The kinetics of enzyme-catalyzed reactions with two or more substrates or products: II. Inhibition: Nomenclature and theory, *Biochim. Biophys. Acta Spec. Sect. Enzymol. Subj.* **67**, 173 (1963).
- [25] R. A. Copeland, *Evaluation of Enzyme Inhibitors in Drug Discovery: A Guide for Medicinal Chemists and Pharmacologists* (Wiley-Interscience, Hoboken, NJ, 2005).

- [26] A. Cornish-Bowden, *Fundamentals of Enzyme Kinetics* (Wiley-VCH, Weinheim, 2012).
- [27] At steady state, total inflow to a node must equal the total outflow (Kirchhoff's current law). Thus, the set of edge net flows is degenerate, and the linearly independent basis is cyclic, known as the fundamental cycle fluxes. It can be constructed by first identifying a spanning tree and then adding back the rest of the edges (chords) one by one to form each of the fundamental cycles. See Refs. [17,49,50] and Fig. S1 of the Supplemental Material [35].
- [28] U. Seifert, Stochastic thermodynamics: From principles to the cost of precision, *Phys. A* **504**, 176 (2018).
- [29] C. Maes, Local detailed balance, *SciPost Phys. Lect. Notes* **32** (2021).
- [30] G. Falasco and M. Esposito, Local detailed balance across scales: From diffusions to jump processes and beyond, *Phys. Rev. E* **103**, 042114 (2021).
- [31] J. A. Owen, T. R. Gingrich, and J. M. Horowitz, Universal thermodynamic bounds on nonequilibrium response with biochemical applications, *Phys. Rev. X* **10**, 011066 (2020).
- [32] G. F. Martins and J. M. Horowitz, Topologically constrained fluctuations and thermodynamics regulate nonequilibrium response, *Phys. Rev. E* **108**, 044113 (2023).
- [33] T. Aslyamov and M. Esposito, Nonequilibrium response for Markov jump processes: Exact results and tight bounds, *Phys. Rev. Lett.* **132**, 037101 (2024).
- [34] T. Aslyamov and M. Esposito, General theory of static response for Markov jump processes, *Phys. Rev. Lett.* **133**, 107103 (2024).
- [35] See Supplemental Material at <https://link.aps.org/supplemental/10.1103/c8kc-rklj> for a review of caliber force theory and its response relations, and for the model details, derivations, and algorithms of the F_1 -ATPase, kinetic-proofreading, and enzyme-inhibition examples, which includes Refs. [36,37].
- [36] R. Chetrite and H. Touchette, Nonequilibrium microcanonical and canonical ensembles and their equivalence, *Phys. Rev. Lett.* **111**, 120601 (2013).
- [37] H. Touchette, The large deviation approach to statistical mechanics, *Phys. Rep.* **478**, 1 (2009).
- [38] E. T. Jaynes, The minimum entropy production principle, *Annu. Rev. Phys. Chem.* **31**, 579 (1980).
- [39] S. Pressé, K. Ghosh, J. Lee, and K. A. Dill, Principles of maximum entropy and maximum caliber in statistical physics, *Rev. Mod. Phys.* **85**, 1115 (2013).
- [40] See Sec. I A of the Supplemental Material for our definitions of invertibility versus reversibility [35].
- [41] Y.-J. Yang, C. D. Kocher, and K. A. Dill, Nonequilibrium theory for adaptive systems in varying environments, [arXiv:2506.19018](https://arxiv.org/abs/2506.19018).
- [42] J. D. Mallory, A. B. Kolomeisky, and O. A. Igoshin, Kinetic control of stationary flux ratios for a wide range of biochemical processes, *Proc. Natl. Acad. Sci. USA* **117**, 8884 (2020).
- [43] A. Murugan, D. A. Huse, and S. Leibler, Speed, dissipation, and error in kinetic proofreading, *Proc. Natl. Acad. Sci. USA* **109**, 12034 (2012).
- [44] P. Sartori and S. Pigolotti, Thermodynamics of error correction, *Phys. Rev. X* **5**, 041039 (2015).
- [45] We define “antiproofreading” by the net reversal of the proofreading cycle, which operates via a different physical mechanism than the state-occupancy or speed-driven antiproofreading regimes discussed in Ref. [51].
- [46] R. Rao and L. Peliti, Thermodynamics of accuracy in kinetic proofreading: Dissipation and efficiency trade-offs, *J. Stat. Mech.* (2015) P06001.
- [47] D. Chiuchiu, S. Mondal, and S. Pigolotti, Pareto optimal fronts of kinetic proofreading, *New J. Phys.* **25**, 043007 (2023).
- [48] A. Goetz, J. Barrios, R. Madsen, and P. Dixit, Non-equilibrium strategies for ligand specificity in signaling networks, *eLife* **14**, RP107524 (2025).
- [49] J. Schnakenberg, Network theory of microscopic and macroscopic behavior of master equation systems, *Rev. Mod. Phys.* **48**, 571 (1976).
- [50] T. L. Hill, *Free Energy Transduction and Biochemical Cycle Kinetics* (Springer, New York, 1989).
- [51] A. Murugan, D. A. Huse, and S. Leibler, Discriminatory proofreading regimes in nonequilibrium systems, *Phys. Rev. X* **4**, 021016 (2014).
- [52] Y.-J. Yang, Codes for “Nonequilibrium theory for molecular machine design”, Zenodo, 2026, version v2, doi: [10.5281/zenodo.21776948](https://doi.org/10.5281/zenodo.21776948).