

Physical constraints on the rhythmicity of the biological clock

YeongKyu Lee  and Changbong Hyeon ^{*}*Korea Institute for Advanced Study, Seoul 02455, South Korea*

(Received 29 July 2025; accepted 13 March 2026; published 9 April 2026)

Circadian rhythms in living organisms are temporal orders emerging from biochemical circuits driven out of equilibrium. Here, considering the KaiABC system, a minimal model in the synthetic biology, we study how the oscillation emerges from the circuit made of three Kai proteins and ATP alone. The phase diagram constructed in terms of KaiC and KaiA concentrations reveals a narrowly bounded oscillatory phase, which naturally explains arrhythmia upon protein overexpression. As dictated by the cost-precision trade-offs of the thermodynamic uncertainty relations, the presence of intrinsic noise, amplified in small systems, demands higher free energy cost to achieve greater rhythmic precision. The cost-minimizing condition within the oscillatory phase is found to generate ~ 21 -h rhythm, which is entrained to 24-h environmental signals as long as the forcing amplitude is greater than $\sim 10\%$ of the metabolic rate. An optimal level of intrinsic noise can also induce oscillations even beyond the Hopf bifurcation, effectively expanding the oscillatory phase. Our study clarifies how the physical factors, such as regulatory mechanism, energy cost, and stochastic noise, contribute to the operation of biological clocks.

DOI: [10.1103/PhysRevResearch.8.023033](https://doi.org/10.1103/PhysRevResearch.8.023033)

I. INTRODUCTION

The KaiABC clock in cyanobacteria, made of three core proteins, KaiA, KaiB, and KaiC, is arguably the simplest biochemical circuit for circadian rhythm that can be reconstituted *in vitro* [1,2]. Driven by ~ 15 ATPs per day [3], and regulated by KaiA and KaiB, KaiC protein exhibits self-sustained ~ 24 -h rhythms of change in its phosphorylation state even in the absence of an external periodic driving or transcription-translation feedback (TTF) regulation [1,4].

Experiments, interrogating the structures and interactions of Kai proteins and their mutants, have offered hints on the molecular origin of the rhythmicity that is inherent in the KaiABC system without TTF regulation [1,4,5]. Several theoretical studies, exploiting a set of coupled nonlinear ordinary differential equations (ODEs) with many state variables, have also addressed experimentally observed features, such as temperature compensation and synchronous oscillations in the ensemble via the KaiC-monomer exchange [6–11]. However, a large number of state variables in the existing ODE models and the complexity inherent to the nonlinearity make it highly demanding even to address the basic questions as to the design principles of a single KaiABC clock. For example, under what condition does the self-sustained oscillations emerge from the underlying circuit? How robust is the oscillatory rhythm against the varying biochemical parameters?

Here, to put the KaiABC clock into the perspective of dynamical systems and to gain systems-level insights into

various factors contributing to the generation of circadian rhythm, we employ a greatly simplified ODE model for the phosphorylation states of KaiC hexamer, developed by Rust *et al.* [12]. After describing the model, we perform the fixed point analysis of the ODE model for the KaiABC circuit to characterize its dynamical behavior in the deterministic limit. The resulting dynamical phase diagram of the circuit as a function of KaiA and KaiC concentrations reveals a narrowly bounded oscillatory phase. As dictated by the cost-precision trade-offs of the thermodynamic uncertainty relations (TURs) [13–15], the free energy cost to generate a nonequilibrium process generally constrains the precision of the resulting dynamics subject to the noise [16–19]. Effect of intrinsic noise on the dynamics is unavoidable in cyanobacteria whose size is finite ranging from $\Omega \approx 10^0 \mu\text{m}^3$ to $\Omega \approx 10^6 \mu\text{m}^3$ [20,21]. Quantifying such a relation and employing the uncertainty product factor from TUR as a measure of system size (Ω)-independent free energy cost (dissipation) to generate oscillations amid fluctuations, we identify the energetically most efficient condition for the KaiABC circuit to generate oscillations. Furthermore, we emphasize the effect of intrinsic noise on the aforementioned phase diagram calculated in the deterministic limit. Even in the phase beyond the Hopf bifurcation lacking stable oscillations, the noise with a proper strength can help enhance a rhythmicity through the coherence resonance (CR) [22–24]. By articulating these points for the KaiABC clock, we aim to offer general insights into the design principles underlying biochemical oscillators.

II. MODEL

KaiC is a hexameric protein, each monomer of which contains two phosphorylation sites (threonine 432 and serine 431) [1]. Rust *et al.*'s model [12] assumes an ordered KaiC phosphorylation cycle along with KaiA/KaiB-mediated effective

^{*}Contact author: hyeoncb@kias.re.kr

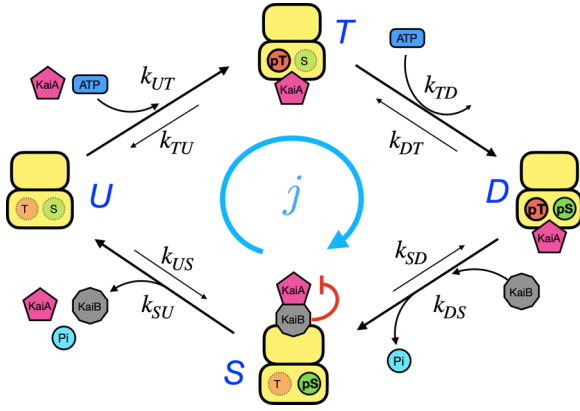


FIG. 1. A kinetic scheme of the KaiC phosphorylation-dephosphorylation cycle for the ODE model studied here (see the main text for the details).

rates, substantially simplifying the complex allosteric dynamics of KaiC hexamer [2,7,10,25–27]. The model, formulated in terms of a set of nonlinear ODEs, is written as $\dot{\mathbf{x}} = \mathbf{F}(\mathbf{x})$ with only three variables $\mathbf{x} = (T, D, S)$:

$$\begin{aligned}\dot{T} &= k_{UT}(S)U + k_{DT}(S)D - [k_{TU}(S) + k_{TD}(S)]T, \\ \dot{D} &= k_{TD}(S)T + k_{SD}(S)S - [k_{DT}(S) + k_{DS}(S)]D, \\ \dot{S} &= k_{US}(S)U + k_{DS}(S)D - [k_{SU}(S) + k_{SD}(S)]S.\end{aligned}\quad (1)$$

Equation (1) represents the coherent evolution of a KaiC hexamer coordinated into four phosphorylation states as depicted in Fig. 1: (1) unphosphorylated state (*U*-KaiC or *U*), (2) threonine-only-phosphorylated state (*T*-KaiC or *T*), (3) serine-only-phosphorylated state (*S*-KaiC or *S*), and (4) serine-threonine-phosphorylated state (*ST*-KaiC or *D*). Since the total concentration of KaiC is conserved without degradation within our relevant time of interest, *U* in Eq. (1) is replaced with $U = [\text{KaiC}] - T - D - S$. Catalyzed by KaiA, the cycle of KaiC (Fig. 1) begins with the phosphorylation of Thr432 (red circle, $T \rightarrow pT$), followed by the secondary phosphorylation at Ser431 (green circle, $S \rightarrow pS$) [28,29]. Upon double phosphorylation, the KaiC undergoes a conformational change and allows KaiB to bind and sequester KaiA, which induces the dephosphorylations of Thr432 and Ser431, and resets the cycle [12].

In Eq. (1), the *S*-dependent transition rate from a state *X* to *Y* is modeled as

$$k_{XY}(S) = k_{XY}^0 + \frac{k_{XY}^A A(S)}{K_{1/2} + A(S)}, \quad (2)$$

where $A(S) = \max(0, [\text{KaiA}] - 2S)$ corresponds to the concentration of free KaiA with the numerical factor 2 reflecting the 2:1 stoichiometry of the interaction between KaiA dimer and KaiC hexamer [30]. k_{XY}^0 is the basal rate of transition and k_{XY}^A in the second term denotes the maximal effect of KaiA on the rate constant. $K_{1/2}$ is the binding affinity between KaiA and KaiC. The expression of $k_{XY}(S)$ with k_{UT}^A , $k_{TD}^A > 0$ models the KaiA-mediated positive regulation for phosphorylations, whereas $k_{XY}(S)$ with k_{DS}^A , $k_{SU}^A < 0$ effectively models the KaiB-mediated KaiA sequestration [31], which suppresses the KaiC autophosphorylation and activates

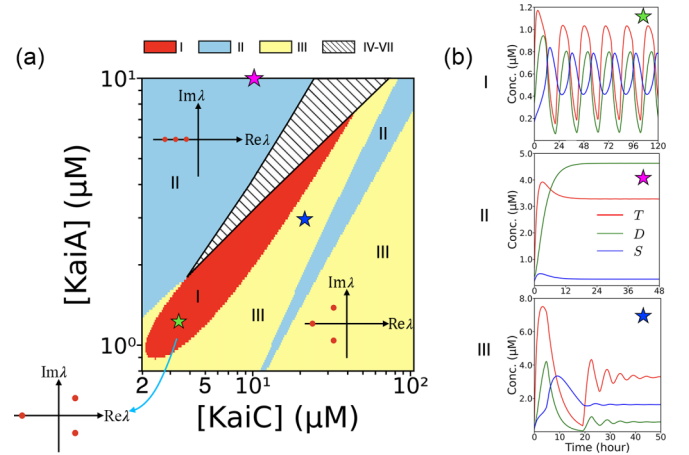


FIG. 2. Dynamical phase diagram and trajectories from the model of KaiABC clock. (a) Phases I–III classified based on the eigenvalue characteristics of the fixed point. More complicated structure of Phases IV–VII is explained in the Supplemental Material [32]. (b) Trajectories of *T*, *D*, and *S* states of KaiC hexamer generated at $([\text{KaiC}](\mu\text{M}), [\text{KaiA}](\mu\text{M})) = (3.4, 1.4)$ (green star) in Phase I, at $(10, 10)$ (magenta star) in Phase II, and at $(20, 3)$ (blue star) in Phase III.

the dephosphorylation process. Thus, the KaiB regulation, accompanied with the accumulation of *S*-KaiC, is implicitly included in the model. The feedback mechanism arising from KaiA sequestration [31] is incorporated into $k_{XY}(S)$ in Eq. (2), such that it enhances synchronization when a phase delay is present between oscillators (see Fig. S1 in the Supplemental Material [32] for the synchronization dynamics of two out-of-phase KaiABC oscillators upon mixing).

The explicit values of all the rate constants involving Eq. (1) such as k_{XY}^0 and k_{XY}^A , determined as the best-fit parameters against experimental measurements [12], are given in Table S1 in the Supplemental Material [32].

III. DYNAMICAL PHASE DIAGRAM

The ODE model of KaiABC in Eq. (1) represents the time evolution of KaiC phosphorylation states at the deterministic limit, i.e., at $\Omega \rightarrow \infty$. To explore the full range of its dynamical behaviors, we vary $[\text{KaiC}]$ and $[\text{KaiA}]$, while assuming that the other parameters associated with the catalysis and protein-protein interactions, i.e., k_{XY}^0 , k_{XY}^A , and $K_{1/2}$, are difficult to tune and held fixed. The linear stability analysis around the fixed points, $\mathbf{x}^* = (T^*, D^*, S^*)$ that satisfy $\mathbf{F}(\mathbf{x}^*) = 0$ in the range of $0 \leq T^*, D^*, S^* \leq [\text{KaiC}]$ (see the Supplemental Material [32]), yields the dynamical phase diagram [Fig. 2(a)].

(1) Phase I is characterized by a single unstable fixed point whose Jacobian matrix has one negative real eigenvalue ($\lambda_1 \in \mathbb{R}_{<0}$) and a pair of complex conjugate eigenvalues with positive real part ($\lambda_{2,3} = \alpha \pm i\beta$ with $\alpha > 0$). At $[\text{KaiC}] = 3.4 \mu\text{M}$ and $[\text{KaiA}] = 1.3 \mu\text{M}$ [green star in Fig. 2(a)], which corresponds to the cost-minimizing point discussed later, the trajectories of three phosphorylated states, namely, *T*, *D*, and *S*, exhibit stable periodic oscillations of $T_{\text{os}} \sim 21$ h with phase lags [Fig. 2(b-I)].

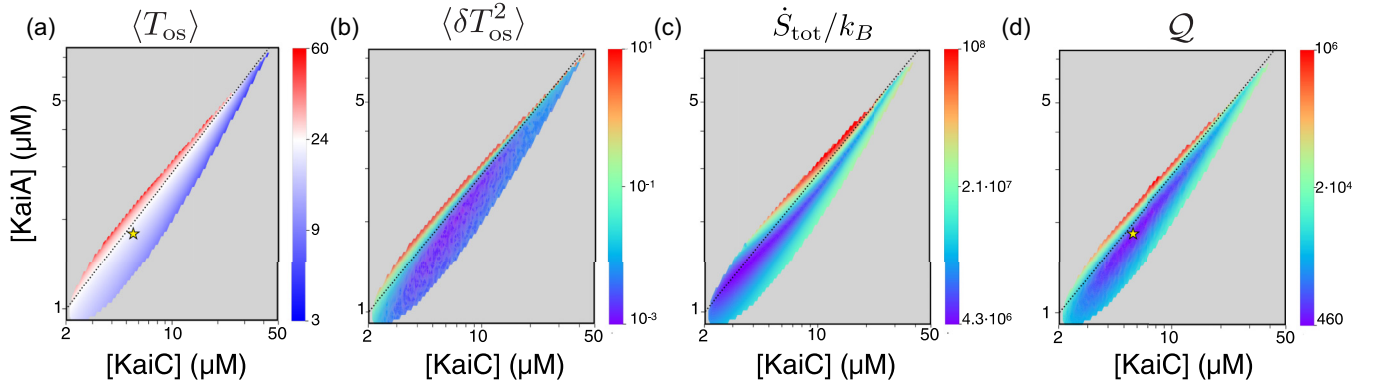


FIG. 3. Dynamics in the oscillatory phase (Phase I) produced at a typical cell volume of cyanobacterium, $\Omega = 1000 \mu\text{m}^3$. (a) Period of oscillation and (b) its variance, (c) entropy production, and (d) the uncertainty product. Note that the magnitudes of $\langle\delta T_{\text{os}}^2\rangle$ and $\dot{S}_{\text{tot}}/k_B$ change with Ω as $\langle\delta T_{\text{os}}^2\rangle \sim 1/\Omega$ and $\dot{S}_{\text{tot}}/k_B \sim \Omega$ (see Fig. S8). The yellow stars in Figs. 3(a) and 3(d) mark the Q -minimizing condition $[\text{KaiC}] = 5.71 \mu\text{M}$ and $[\text{KaiA}] = 1.87 \mu\text{M}$, which leads to $\langle T_{\text{os}} \rangle \simeq 21 \text{ h}$ in panel (a).

(2) Phases II and III are characterized by a single stable fixed point, but each has different eigenvalue structure. For Phase II, $\lambda_{1,2,3} \in \mathbb{R}_{<0}$, whereas for Phase III, $\lambda_1 \in \mathbb{R}_{<0}$ and $\lambda_{2,3} = \alpha \pm i\beta$ with $\alpha \in \mathbb{R}_{<0}$. Trajectories generated in these phases (II and III) always converge to a stable fixed point; yet due to the difference in the eigenvalue structure, the convergence to the corresponding fixed point in Phase III is preceded by damped oscillations [blue star, Fig. 2(b-III)], whereas the trajectories in Phase II exhibit direct damping without oscillation [magenta star, Fig. 2(b-II)].

The remaining phases demarcated by the hashed lines are characterized by three fixed points of various types, giving rise to complicated dynamics that requires more careful analysis. However, due to the presence of, at least, a single stable fixed point, trajectories in this region are convergent in the presence of noise at $t \rightarrow \infty$ (see text and Fig. S2 in the Supplemental Material [32]).

Notably, Phase I is the only oscillatory phase. A few points are noteworthy. (1) The narrowly bounded oscillatory phase over the range of $[\text{KaiA}]$ and $[\text{KaiC}]$ straightforwardly accounts for experimentally observed arrhythmia (loss of rhythmicity or damping) when Kai proteins are either overexpressed or deleted from the system [4,12,25,33–36]. Such sensitivity points to the importance of tightly regulated expression levels of KaiABC operon for maintaining the rhythmicity. (2) For fixed $[\text{KaiA}]$, the amplitude of oscillations changes nonmonotonically with $[\text{KaiC}]$ or $\langle T_{\text{os}} \rangle$ [see Figs. S3(a) and S3(b) in the Supplemental Material [32]], getting greater when $[\text{KaiA}]$ and $[\text{KaiC}]$ are higher [Fig. S3(a) in the Supplemental Material [32]]. (3) Consistent with experiments [1,25,26,37–40], mutations that affect either the autokinase activity of KaiC or the binding affinity between KaiA and KaiC can alter the period of oscillations. Such effects can be incorporated to the current model by tuning the phosphorylation rates (k_{UT} and k_{TD}) or the binding constant ($K_{1/2}$) in Eqs. (1) and (2) (see Fig. S4 in the Supplemental Material [32] for trajectories with altered periodicity and amplitude, and text and Fig. S5 in the Supplemental Material [32] for the changes in the corresponding phase diagrams).

IV. DISSIPATION CONSTRAINS THE REGULARITY OF OSCILLATORY DYNAMICS

While high-dimensional dynamical systems may display complex behavior, suitable regulatory mechanisms can confine their long-term dynamics to a two-dimensional (2D) invariant manifold exhibiting a limit cycle [41]. The mean period of oscillation ($\langle T_{\text{os}} \rangle$) calculated in Phase I indicates that the 24-h cycle is formed along the narrow parameter space, indicating a tight regulation of the clock (see Fig. S6 for the detailed view of the heat map of $\langle T_{\text{os}} \rangle$). From both the numerical analysis of Eq. (1) and its reduction to the Stuart-Landau form [41] (see text in the Supplemental Material [32]), we find a relation $[\text{KaiA}] \propto [\text{KaiC}]^\alpha$ with $\alpha \approx 2/3$ satisfying the locus of $\langle T_{\text{os}} \rangle \simeq 24 \text{ h}$ in the heat map over the range of $[\text{KaiA}] \approx (1\text{--}3) \mu\text{M}$ and $[\text{KaiC}] \approx (2\text{--}10) \mu\text{M}$ [dotted line in Fig. 3(a); see also Figs. S6 and S7]. This is, in effect, consistent with the 1/3 KaiA to KaiC ratio for *in vitro* oscillations [36].

Noting that the KaiABC clock is a nonequilibrium device exhibiting self-sustained oscillations under a constant drive of ATP, we put its rhythmic operation into the perspective of thermodynamic uncertainty relation (TUR) and assess its thermodynamic optimality [42–44]. In particular, we adapt the TUR for the first passage time processes [45] and employ the relation to assess the cost-precision trade-off and identify the cost-minimizing condition for the operation of stochastic clock [14,46]. The uncertainty product Q of TUR for a stochastic clock is written as [46]

$$Q = \frac{\Delta S_{\text{cyc}} \langle\delta T_{\text{os}}^2\rangle}{k_B \langle T_{\text{os}} \rangle^2} = \frac{\dot{S}_{\text{tot}} \langle\delta T_{\text{os}}^2\rangle}{k_B \langle T_{\text{os}} \rangle}, \quad (3)$$

where ΔS_{cyc} is the entropy production per clock cycle, the mean and variance of oscillatory period are given by $\langle T_{\text{os}} \rangle$ and $\langle\delta T_{\text{os}}^2\rangle$, respectively, and $\dot{S}_{\text{tot}} = \Delta S_{\text{cyc}}/\langle T_{\text{os}} \rangle$ denotes the mean entropy production rate. Equation (3) dictates that for a given Q , the cost to operate the clock (ΔS_{cyc}) is counterbalanced with the relative error in the period ($\langle\delta T_{\text{os}}^2\rangle^{1/2}/\langle T_{\text{os}} \rangle$). The value of dimensionless factor Q , which varies with the clock “design” (parameters, network topology, and regulatory mechanism of the biochemical circuit), is used to assess the efficiency of the clock by linking irreversibility

to measurable fluctuations [42,43] or to the power efficiency (η = output power/input power) of stochastic engines or molecular motors [15,47,48]. A stochastic clock with a smaller Q value can be considered better designed in terms of the precision and energy expenditure. For dynamical processes exhibiting stochastic oscillations under *constant* driving, Q is bounded as $Q \geq Q_{\min}^{\text{univ}} = 2$ [49]. For a type of cyclic network (Fig. 1), the universal bound is achieved close to equilibrium [13,49,50], although the KaiABC circuit would cease to oscillate in such a condition. Thus, our objective here is to identify the condition that minimizes Q within the oscillatory phase.

Evaluation of Q in Eq. (3) is straightforward. The network of KaiC cycle depicted in Fig. 1 is none other than a reversible unicyclic network. The mean entropy production rate $\dot{S}_{\text{tot}}$ in Eq. (3) is given by

$$\dot{S}_{\text{tot}}/k_B = (j_+ - j_-) \ln \left(\frac{j_+}{j_-} \right) \times [\text{KaiC}] \times \Omega, \quad (4)$$

where $j_+ = \bar{k}_{UT}\bar{k}_{TD}\bar{k}_{DS}\bar{k}_{SU}/\Sigma$ and $j_- = \bar{k}_{US}\bar{k}_{SD}\bar{k}_{DT}\bar{k}_{TU}/\Sigma$ with $\Sigma = \bar{k}_{UT}\bar{k}_{TD}\bar{k}_{DS} + \bar{k}_{TD}\bar{k}_{DS}\bar{k}_{SU} + \bar{k}_{DS}\bar{k}_{SU}\bar{k}_{UT} + \bar{k}_{SU}\bar{k}_{UT}\bar{k}_{TD} + \bar{k}_{DT}\bar{k}_{SD}\bar{k}_{US} + \bar{k}_{SD}\bar{k}_{US}\bar{k}_{TU} + \bar{k}_{US}\bar{k}_{TU}\bar{k}_{DT} + \bar{k}_{TU}\bar{k}_{DT}\bar{k}_{SD}$ [51]. Here, it should be noted that $\bar{k}_{XY}$ denotes the rate constant averaged over the cycle period at cyclic steady states, i.e., $\bar{k}_{XY} = (1/T) \int_0^T k_{XY}(\tau) d\tau$. The rate constants in Eq. (1) are the functions of [KaiA] and [KaiC], and so are both the steady-state current $j = \bar{k}_{XY}X^{ss} - \bar{k}_{YX}Y^{ss} = j_+ - j_-$ (Fig. 1) and the entropy production [Eq. (4)]. Note that the entropy production is an extensive quantity that increases with the number of KaiC proteins in the system [$N_{\text{KaiC}} = [\text{KaiC}] \times \Omega$, Eq. (4)], i.e., $\dot{S}_{\text{tot}} \propto \Omega$, which is compensated by $\langle \delta T_{\text{os}}^2 \rangle \propto \Omega^{-1}$ in the uncertainty product. $\langle T_{\text{os}} \rangle$, determined by the dynamics confined in a 2D invariant manifold around the unstable fixed point, is independent of Ω (see Fig. S8). Taken together, the uncertainty product Q defined in Eq. (3) is an Ω -independent measure.

When [KaiA] and [KaiC] are varied while holding all other parameters involving catalytic power and protein-protein interactions (Table S1), the Q -minimizing condition KaiABC clock with $Q_{\min} \simeq 460$ is formed at [KaiC] = 5.71 μM and [KaiA] = 1.82 μM , yielding the oscillatory period of $\langle T_{\text{os}} \rangle \simeq 21$ h [Figs. 3(a) and 3(d)]. Similarly to error-correcting biochemical processes with large Q values that have to expend large free energy [15], the KaiABC clock, severely underperforming the universal TUR bound ($Q_{\min} \simeq 460 \gg Q_{\min}^{\text{univ}} = 2$), might be considered inefficient. Nevertheless, it should be noted that in order to create a temporal order from a given biochemical circuit, a finite amount of free energy consumption, which necessarily drives the system away from the detailed balance condition corresponding to $Q = Q_{\min}^{\text{univ}} = 2$, is inevitable. For the case of KaiABC system, the cost-minimizing point to create an oscillation is formed at $Q = Q_{\min} \simeq 460$ within the oscillatory phase, when [KaiA] and [KaiC] are the only two variables.

Figure S5 demonstrates a case in which $Q_{\min}$ is slightly reduced ($Q_{\min} \simeq 315$) upon increasing the binding affinity ($K_{1/2} = 0.43 \rightarrow 0.35$ μM , Fig. S5d). In other cases, either the area of oscillatory phase shrinks [Figs. S5(a) and S5(e) in

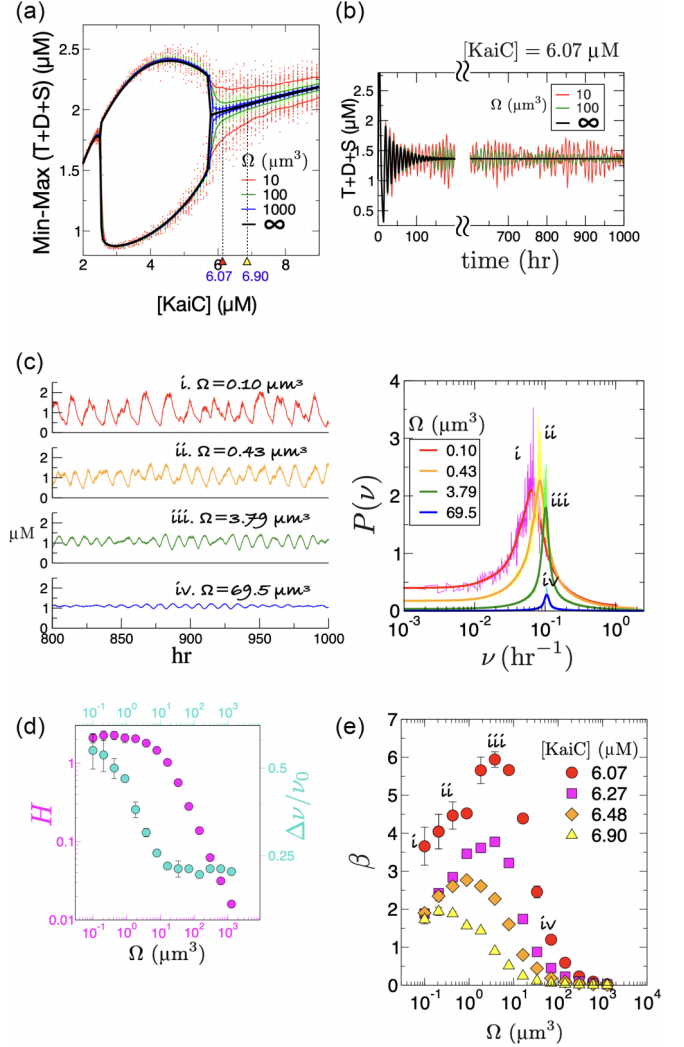


FIG. 4. Noise-induced oscillation. (a) Bifurcation diagram at [KaiA] = 1.3 μM . Solid black line, blue, green, and red dots depict the minimum and the maximum values of $(T + D + S)$ for $\Omega \rightarrow \infty, 1000, 100$, and 10 μm^3 , respectively. (b) Time evolutions of $(T + D + S)$ at [KaiC] = 6.07 μM . (c) Noise-induced oscillations generated at [KaiC] = 6.07 μM for varying Ω 's: (i) $\Omega = 0.10$, (ii) 0.43, (iii) 3.79, and (iv) 69.5 μm^3 (left) and the corresponding power spectra $P(\nu)$ s (right). (d) Height (H) and width ($\Delta\nu/\nu_0$) of the resonant peak vs Ω . (e) SNR ($\beta = H/(\Delta\nu/\nu_0)$) vs Ω for varying [KaiC]. The values of β calculated at different Ω 's in panel (c) are marked with (i) to (iv).

the Supplemental Material [32]], or increases of the area are accompanied with a significant increase of $Q_{\min}$ [Figs. S5(b) and S5(c) in the Supplemental Material [32]].

V. NOISE-INDUCED TEMPORAL ORDER

When [KaiC] is varied over 2–10 μM at [KaiA] = 1.3 μM , passing through Phase I [Fig. 2(a)], the stability of the fixed point undergoes a transition. Two supercritical Hopf bifurcation points, [KaiC]_{cr} = 2.55 μM and 5.71 μM , are identified in the bifurcation diagram calculated at the deterministic limit, namely, at $\Omega \rightarrow \infty$ [Fig. 4(a)]. Trajectories generated beyond the Hopf bifurcation point ([KaiC] = 6.07 μM), where

deterministic oscillations are expected to vanish, display noisy oscillations at $\Omega = 10$ (red) and $100 \mu\text{m}^3$ (green) [Fig. 4(b)]. The minimum and maximum values of the amplitude overlaid on the diagram blur the sharp boundary obtained at $\Omega \rightarrow \infty$ [black line, Fig. 4(a)].

To study the effect of noise, whose intensity changes with the system size as $\propto 1/\sqrt{\Omega}$, more systematically, we examine the power spectra $P(\nu) = \int C_{xx}(\tau) e^{-2\pi i \nu \tau} d\tau$, the Fourier transform of autocorrelation function at steady state $C_{xx}(\tau) = \frac{1}{T} \int_0^T x(t)x(t+\tau)dt$ with $x(t) = (T+D+S)(t)$, for varying Ω at $[\text{KaiC}] = 6.07 \mu\text{M}$ [Figs. 4(b) and 4(c)]. A resonant peak formed in $P(\nu)$ at $\nu = \nu_0 \sim 10^{-1} \text{ h}^{-1}$ points to the ~ 10 -h rhythmicity in the trajectories [Fig. 4(c)]. The height (H) and width ($\Delta\nu/\nu_0$) of the resonant peak in $P(\nu)$ increase monotonically with the noise level [Fig. 4(d)], which are consistent with those previously discovered near supercritical Hopf bifurcation in Ref. [24]. For $[\text{KaiC}] = 6.07 \mu\text{M}$, the signal-to-noise ratio (SNR) of the resonant peak, i.e., the regularity of oscillations in time domain, quantified in terms of $\beta = H/(\Delta\nu/\nu_0)$ is maximized at an intermediate system size $\Omega_{\text{max}} = \arg \max_{\Omega} \beta \simeq 4 \mu\text{m}^3$ [Fig. 4(e)], which signifies that there is an optimal noise intensity (or Ω value) that yields the maximal regularity in oscillations (equivalently, the maximal SNR of the signal in frequency space). Small noise added to a stable trajectory is ineffective to induce oscillations, whereas large noise is also expected to hinder generation of oscillations with a regular period. When $[\text{KaiC}]$ is varied away from the Hopf-bifurcation point ($[\text{KaiC}]_{\text{cr}} = 5.71 \mu\text{M}$), the optimal noise level for the resonance shifts toward the smaller Ω [see the peak positions in Fig. 4(e)], suggesting that stronger noise is required to compensate for the increased distance from the bifurcation. Our finding that an optimal level of intrinsic noise can induce temporal order in a trajectory that is otherwise stable and nonoscillatory corresponds to the *coherence resonance* or *stochastic resonance without periodic force* [22] that was also discovered for excitable systems [23,52].

VI. DISCUSSION

Consistent with the existing experimental reports [4,12,25,33–36], the phase diagram revealed in this study clarifies that the oscillatory phase of the KaiABC is tightly regulated over the relatively narrow range of biologically tunable parameters, $[\text{KaiA}]$ and $[\text{KaiC}]$. This is contrasted with other biochemical circuits, such as the quorum sensing model of repressilator [54], that give rise to a broad oscillatory phase under double-negative feedback loops [55]. For the KaiABC clock emerging from the circuit made of three Kai proteins without TTF regulation, the parameter space for timekeeping is sensitively balanced by the competing actions between the positive and negative feedbacks of KaiA and KaiB.

Within the oscillatory phase, the parameter space $\mathbf{c} = ([\text{KaiC}], [\text{KaiA}])$ giving rise to $\langle T_{\text{os}} \rangle \simeq 24 \text{ h}$ [dotted line in Fig. 3(a)] and the condition $\mathbf{c}^* = ([\text{KaiC}]^*, [\text{KaiA}]^*)$ for the cost-minimizing period $\langle T_{\text{os}} \rangle(\mathbf{c}^*) \simeq 21 \text{ h}$ are distinct; yet, their separation in time is not significant. For an external forcing with 24-h periodicity, it is expected that the 21-h oscillation is entrained to the external cycle as long as the forcing amplitude is large enough [56–59]. The stable entrainment occurs,

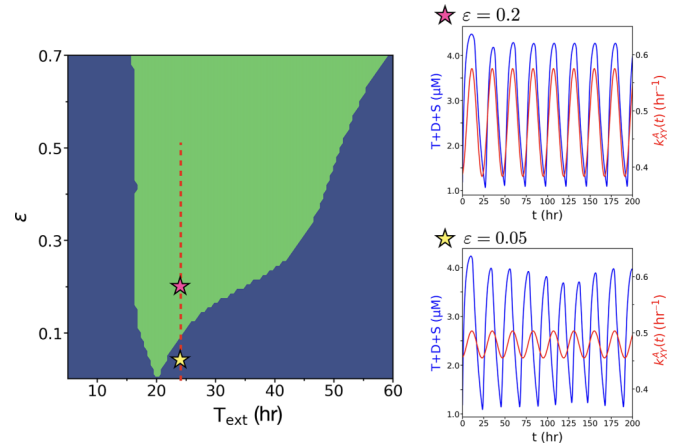


FIG. 5. Entrainment of the ~ 21 -h KaiABC rhythm at the cost-minimizing point to 24-h environmental cues. Based on Ref. [53] suggesting light-driven changes in metabolism, we assume a modulation of KaiA-associated kinetic rate defined in Eq. (2): $k_{XY}^A \rightarrow k_{XY}^A [1 + \varepsilon \sin(2\pi t/T_{\text{ext}})]$. In the diagram of external forcing amplitude (ε) vs. its period (T_{ext}), the entrainment of the intrinsic oscillation to the 24-h cycle occurs in the regime depicted in green, corresponding to the *Arnold tongue*. An increasing ε at $T_{\text{ext}} = 24$, depicted with the red dashed line, indicates that the entrainment occurs when ε is greater than 10% of the intrinsic reaction rate (k_{XY}^A). Shown on the right is the entrainment of $T+D+S$ dynamics (blue) under external periodic forcing (red) at $\varepsilon = 0.2$ (top panel), contrasted with the absence of entrainment at $\varepsilon = 0.05$ (bottom panel).

effectively putting the system into the “Arnold tongue,” when the forcing amplitude of sinusoidal perturbation (ε) added to the KaiA-associated metabolic rate is greater than 10% of metabolic rate, i.e., $\varepsilon \gtrsim 0.1$ (see Fig. 5 and its caption).

Our study on the minimal biochemical oscillator suggests that a large Q factor, signifying large free energy dissipation, is inevitable for generation of oscillatory dynamics from biochemical circuits. Our finding that substantial amount of ATP per cycle has to be expended to create an oscillation even from a simplistic biochemical circuit, in fact, aligns well with other biophysical machineries, such as molecular motors [15,43,48,60], biological error correction [61–63], molecular chaperones [64,65], and pattern formation during the early stage of embryogenesis [66,67].

The results from this study on KaiABC clock are general, not necessarily specific to the Rust *et al.*’s model. The dynamic quantities required for calculating the uncertainty product ($\mathcal{Q}$), i.e., $\langle T_{\text{os}} \rangle$, $\langle \delta T_{\text{os}}^2 \rangle$, and $\dot{S}_{\text{tot}}/k_B$, can be quantified entirely based on the experimentally measured time traces. For given dynamical trajectories, the period and its variance are straightforward to quantify, although extracting an accurate value of entropy production based on Eq. (4) may involve a certain degree of subtlety, partly due to the level of coarse graining that the Rust *et al.*’s model adopts. We assume that the trajectories generated under different conditions (e.g., at different values of $[\text{KaiC}]$ and $[\text{KaiA}]$) are reasonable extrapolations of the model whose kinetic parameters are decided against the experimental measurement (Table S1).

The cost-optimal rhythmicity and the noise-induced temporal order revealed in this study suggest how physics

constrains the operation of stochastic clock in cells that emerges from biochemical circuit. Under the hood of dynamical systems [57] and stochastic thermodynamics [68], these dynamical features can be regarded generic to biological clocks. They can, in principle, be uncovered in more complicated biochemical oscillators upon phase reduction and center manifold reduction of the associated dynamics [69,70]. Our study dissecting the capacity of biochemical circuits provides concrete physical insights into the principles governing self-sustained biological clocks and can potentially be extended to shed lights on the bioenergetics at the cellular scale [71–73] and beyond [74,75]. Building on earlier efforts to construct synthetic gene oscillators with tunable or self-sustained dynamics [76–78],

our findings can be of great use in guiding the rational design of synthetic oscillators.

ACKNOWLEDGMENT

We acknowledge the support from a KIAS individual Grants No. CG097901 (Y.L.) and No. CG035003 (C.H.) at the Korea Institute for Advanced Study. We thank the Center for Advanced Computation in KIAS for providing the computing resources.

DATA AVAILABILITY

No data were created or analyzed in this study.

- [1] M. Nakajima, K. Imai, H. Ito, T. Nishiwaki, Y. Murayama, H. Iwasaki, T. Oyama, and T. Kondo, Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation *in vitro*, *Science* **308**, 414 (2005).
- [2] H. Kageyama, T. Nishiwaki, M. Nakajima, H. Iwasaki, T. Oyama, and T. Kondo, Cyanobacterial circadian pacemaker: Kai protein complex dynamics in the KaiC phosphorylation cycle *in vitro*, *Mol. Cell* **23**, 161 (2006).
- [3] K. Terauchi, Y. Kitayama, T. Nishiwaki, K. Miwa, Y. Murayama, T. Oyama, and T. Kondo, ATPase activity of KaiC determines the basic timing for circadian clock of cyanobacteria, *Proc. Natl. Acad. Sci. USA* **104**, 16377 (2007).
- [4] M. Ishiura, S. Kutsuna, S. Aoki, H. Iwasaki, C. R. Andersson, A. Tanabe, S. S. Golden, C. H. Johnson, and T. Kondo, Expression of a gene cluster kaiABC as a circadian feedback process in cyanobacteria, *Science* **281**, 1519 (1998).
- [5] J. A. Swan, C. R. Sandate, A. G. Chavan, A. M. Freeberg, D. Etwaru, D. C. Ernst, J. G. Palacios, S. S. Golden, A. LiWang, G. C. Lander, and C. L. Partch, Coupling of distant ATPase domains in the circadian clock protein KaiC, *Nat. Struct. Mol. Biol.* **29**, 759 (2022).
- [6] E. Emberly and N. S. Wingreen, Hourglass model for a protein-based circadian oscillator, *Phys. Rev. Lett.* **96**, 038303 (2006).
- [7] J. S. van Zon, D. K. Lubensky, P. R. Altena, and P. R. ten Wolde, An allosteric model of circadian KaiC phosphorylation, *Proc. Natl. Acad. Sci. USA* **104**, 7420 (2007).
- [8] T. S. Hatakeyama and K. Kaneko, Generic temperature compensation of biological clocks by autonomous regulation of catalyst concentration, *Proc. Natl. Acad. Sci. USA* **109**, 8109 (2012).
- [9] M. Sasai, Role of the reaction-structure coupling in temperature compensation of the KaiABC circadian rhythm, *PLoS Comput. Biol.* **18**, e1010494 (2022).
- [10] D. Zhang, Y. Cao, Q. Ouyang, and Y. Tu, The energy cost and optimal design for synchronization of coupled molecular oscillators, *Nat. Phys.* **16**, 95 (2020).
- [11] H. Fu, C. Fei, Q. Ouyang, and Y. Tu, Temperature compensation through kinetic regulation in biochemical oscillators, *Proc. Natl. Acad. Sci. USA* **121**, e2401567121 (2024).
- [12] M. J. Rust, J. S. Markson, W. S. Lane, D. S. Fisher, and E. K. O'Shea, Ordered phosphorylation governs oscillation of a three-protein circadian clock, *Science* **318**, 809 (2007).
- [13] A. C. Barato and U. Seifert, Thermodynamic uncertainty relation for biomolecular processes, *Phys. Rev. Lett.* **114**, 158101 (2015).
- [14] R. Marsland III, W. Cui, and J. M. Horowitz, The thermodynamic uncertainty relation in biochemical oscillations, *J. R. Soc. Interface* **16**, 20190098 (2019).
- [15] Y. Song and C. Hyeon, Thermodynamic uncertainty relation to assess biological processes, *J. Chem. Phys.* **154**, 130901 (2021).
- [16] P. Mehta and D. J. Schwab, Energetic costs of cellular computation, *Proc. Natl. Acad. Sci. USA* **109**, 17978 (2012).
- [17] Y. Cao, H. Wang, Q. Ouyang, and Y. Tu, The free-energy cost of accurate biochemical oscillations, *Nat. Phys.* **11**, 772 (2015).
- [18] P. Sartori and Y. Tu, Free energy cost of reducing noise while maintaining a high sensitivity, *Phys. Rev. Lett.* **115**, 118102 (2015).
- [19] G. Lan, P. Sartori, S. Neumann, V. Sourjik, and Y. Tu, The energy-speed-accuracy trade-off in sensory adaptation, *Nat. Phys.* **8**, 422 (2012).
- [20] E. Marañón, C. Fernández-González, and G. A. Tarran, Effect of temperature, nutrients and growth rate on picophytoplankton cell size across the atlantic ocean, *Sci. Rep.* **14**, 28034 (2024).
- [21] A. Hahn, M. Stevanovic, O. Mirus, and E. Schleiff, The TolC-like protein HgdD of the cyanobacterium *Anabaena* sp. PCC₇₁₂₀ is involved in secondary metabolite export and antibiotic resistance, *J. Biol. Chem.* **287**, 41126 (2012).
- [22] H. Gang, T. Ditzinger, C.-Z. Ning, and H. Haken, Stochastic resonance without external periodic force, *Phys. Rev. Lett.* **71**, 807 (1993).
- [23] A. S. Pikovsky and J. Kurths, Coherence resonance in a noise-driven excitable system, *Phys. Rev. Lett.* **78**, 775 (1997).
- [24] O. Ushakov, H.-J. Wünsche, F. Henneberger, I. Khovanov, L. Schimansky-Geier, and M. Zaks, Coherence resonance near a Hopf bifurcation, *Phys. Rev. Lett.* **95**, 123903 (2005).
- [25] H. Iwasaki, T. Nishiwaki, Y. Kitayama, M. Nakajima, and T. Kondo, KaiA-stimulated KaiC phosphorylation in circadian timing loops in cyanobacteria, *Proc. Natl. Acad. Sci. USA* **99**, 15788 (2002).
- [26] Y. Kitayama, H. Iwasaki, T. Nishiwaki, and T. Kondo, KaiB functions as an attenuator of KaiC phosphorylation in the cyanobacterial circadian clock system, *EMBO J.* **22**, 2127 (2003).

- [27] K. Eguchi, M. Yoda, T. P. Terada, and M. Sasai, Mechanism of robust circadian oscillation of KaiC phosphorylation *in vitro*, *Biophys. J.* **95**, 1773 (2008).
- [28] Y. Xu, T. Mori, R. Pattanayek, S. Pattanayek, M. Egli, and C. H. Johnson, Identification of key phosphorylation sites in the circadian clock protein KaiC by crystallographic and mutagenetic analyses, *Proc. Natl. Acad. Sci. USA* **101**, 13933 (2004).
- [29] M. Egli, R. Pattanayek, J. H. Sheehan, Y. Xu, T. Mori, J. A. Smith, and C. H. Johnson, Loop-loop interactions regulate KaiA-stimulated KaiC phosphorylation in the cyanobacterial KaiABC circadian clock, *Biochemistry* **52**, 1208 (2013).
- [30] F. Hayashi, H. Ito, M. Fujita, R. Iwase, T. Uzumaki, and M. Ishiura, Stoichiometric interactions between cyanobacterial clock proteins KaiA and KaiC, *Biochem. Biophys. Res. Commun.* **316**, 195 (2004).
- [31] C. Brettschneider, R. J. Rose, S. Hertel, I. M. Axmann, A. J. Heck, and M. Kollmann, A sequestration feedback determines dynamics and temperature entrainment of the KaiABC circadian clock, *Mol. Syst. Biol.* **6**, 389 (2010).
- [32] See Supplemental Material at <http://link.aps.org/supplemental/10.1103/9byx-f411> for the linear stability analysis and Jacobian matrix for the model, stochastic simulation using Gillespie algorithm, synchronization of two KaiABC clocks upon mixing, dynamics in the phases [IV–VII in Fig. 2(a)] with three fixed points, effects of mutation on the KaiABC dynamics, approximation of limit cycle dynamics in phase I to the Stuart-Landau equation, Figs. S1–S8, and Table S1.
- [33] N. Kawamoto, H. Ito, I. T. Tokuda, and H. Iwasaki, Damped circadian oscillation in the absence of KaiA in *Synechococcus*, *Nat. Commun.* **11**, 2242 (2020).
- [34] Y. Xu, T. Mori, and C. H. Johnson, Circadian clock-protein expression in cyanobacteria: Rhythms and phase setting, *EMBO J.* **19**, 3349 (2000).
- [35] X. Qin, M. Byrne, T. Mori, P. Zou, D. R. Williams, H. Mchaourab, and C. H. Johnson, Intermolecular associations determine the dynamics of the circadian KaiABC oscillator, *Proc. Natl. Acad. Sci. USA* **107**, 14805 (2010).
- [36] M. Nakajima, H. Ito, and T. Kondo, *In vitro* regulation of circadian phosphorylation rhythm of cyanobacterial clock protein KaiC by KaiA and KaiB, *FEBS Lett.* **584**, 898 (2010).
- [37] Y.-G. Chang, N.-W. Kuo, R. Tseng, and A. LiWang, Flexibility of the C-terminal, or CII, ring of KaiC governs the rhythm of the circadian clock of cyanobacteria, *Proc. Natl. Acad. Sci. USA* **108**, 14431 (2011).
- [38] K. Ito-Miwa, Y. Furuike, S. Akiyama, and T. Kondo, Tuning the circadian period of cyanobacteria up to 6.6 days by the single amino acid substitutions in KaiC, *Proc. Natl. Acad. Sci. USA* **117**, 20926 (2020).
- [39] Y. Ouyang, C. R. Andersson, T. Kondo, S. S. Golden, and C. H. Johnson, Resonating circadian clocks enhance fitness in cyanobacteria, *Proc. Natl. Acad. Sci. USA* **95**, 8660 (1998).
- [40] R. Pattanayek, D. R. Williams, S. Pattanayek, Y. Xu, T. Mori, C. H. Johnson, P. L. Stewart, and M. Egli, Analysis of KaiA–KaiC protein interactions in the cyanobacterial circadian clock using hybrid structural methods, *EMBO J.* **25**, 2017 (2006).
- [41] Y. A. Kuznetsov, *Elements of Applied Bifurcation Theory* (Springer, New York, 1998).
- [42] A. Dechant and S.-I. Sasa, Current fluctuations and transport efficiency for general Langevin systems, *J. Stat. Mech.* (2018) 063209.
- [43] W. Hwang and C. Hyeon, Energetic costs, precision, and transport efficiency of molecular motors, *J. Phys. Chem. Lett.* **9**, 513 (2018).
- [44] J. M. Horowitz and T. R. Gingrich, Thermodynamic uncertainty relations constrain non-equilibrium fluctuations, *Nat. Phys.* **16**, 15 (2020).
- [45] T. R. Gingrich and J. M. Horowitz, Fundamental bounds on first passage time fluctuations for currents, *Phys. Rev. Lett.* **119**, 170601 (2017).
- [46] P. Kim and C. Hyeon, Thermodynamic optimality of glycolytic oscillations, *J. Phys. Chem. B* **125**, 5740 (2021).
- [47] P. Pietzonka, A. C. Barato, and U. Seifert, Universal bound on the efficiency of molecular motors, *J. Stat. Mech.* (2016) 124004.
- [48] V. Rodríguez-Franco, M. Spiering, P. Bianco, F. Ritort, and M. Mañosas, Continuous-time random walk model for the diffusive motion of helicases, *QRB Discovery* **6**, e26 (2025).
- [49] A. C. Barato and U. Seifert, Cost and precision of Brownian clocks, *Phys. Rev. X* **6**, 041053 (2016).
- [50] T. Koyuk and U. Seifert, Operationally accessible bounds on fluctuations and entropy production in periodically driven systems, *Phys. Rev. Lett.* **122**, 230601 (2019).
- [51] D. A. Beard and H. Qian, *Chemical Biophysics: Quantitative Analysis of Cellular Systems* (Cambridge University Press, Cambridge, 2008).
- [52] Q. Li and Y. Wang, Coupling and internal noise sustain synchronized oscillation in calcium system, *Biophys. Chem.* **129**, 23 (2007).
- [53] M. J. Rust, S. S. Golden, and E. K. O'Shea, Light-driven changes in energy metabolism directly entrain the cyanobacterial circadian oscillator, *Science* **331**, 220 (2011).
- [54] J. Garcia-Ojalvo, M. B. Elowitz, and S. H. Strogatz, Modeling a synthetic multicellular clock: Repressilators coupled by quorum sensing, *Proc. Natl. Acad. Sci. USA* **101**, 10955 (2004).
- [55] J. K. Kim and D. B. Forger, A mechanism for robust circadian timekeeping via stoichiometric balance, *Mol. Syst. Biol.* **8**, 630 (2012).
- [56] M. Fang, A. G. Chavan, A. LiWang, and S. S. Golden, Synchronization of the circadian clock to the environment tracked in real time, *Proc. Natl. Acad. Sci. USA* **120**, e2221453120 (2023).
- [57] A. Pikovsky, M. Rosenblum, and J. Kurths, in *Synchronization: A Universal Concept in Nonlinear Science*, edited by B. Chirikov, C. Predrag, F. Moss, and H. Swinney (Cambridge University Press, Cambridge, 2002).
- [58] M. L. Heltberg, S. Krishna, L. P. Kadanoff, and M. H. Jensen, A tale of two rhythms: Locked clocks and chaos in biology, *Cell Syst.* **12**, 291 (2021).
- [59] P. Kim and C. Hyeon, Glycolytic oscillations under periodic drivings, *J. R. Soc. Interface* **21**, 20230588 (2024).
- [60] C. Maggi, F. Saglimbeni, V. C. Sosa, R. Di Leonardo, B. Nath, and A. Puglisi, Thermodynamic limits of sperm swimming precision, *PRX Life* **1**, 013003 (2023).
- [61] A. Murugan, D. A. Huse, and S. Leibler, Speed, dissipation, and error in kinetic proofreading, *Proc. Natl. Acad. Sci. USA* **109**, 12034 (2012).
- [62] Y. Song and C. Hyeon, Thermodynamic cost, speed, fluctuations, and error reduction of biological copy machines, *J. Phys. Chem. Lett.* **11**, 3136 (2020).

- [63] J. D. Mallory, O. A. Igoshin, and A. B. Kolomeisky, Do we understand the mechanisms used by biological systems to correct their errors? *J. Phys. Chem. B* **124**, 9289 (2020).
- [64] I. Jarmoskaite, P. Tijerina, and R. Russell, ATP utilization by a DEAD-box protein during refolding of a misfolded group I intron ribozyme, *J. Biol. Chem.* **296**, 100132 (2021).
- [65] Y. Song, D. Thirumalai, and C. Hyeon, Moderate activity of RNA chaperone maximizes the yield of self-spliced pre-RNA *in vivo*, *Proc. Natl. Acad. Sci. USA* **119**, e2209422119 (2022).
- [66] Y. Song and C. Hyeon, Cost-precision trade-off relation determines the optimal morphogen gradient for accurate biological pattern formation, *eLife* **10**, e70034 (2021).
- [67] D. Zhang, C. Zhang, Q. Ouyang, and Y. Tu, Free energy dissipation enhances spatial accuracy and robustness of self-positioned Turing pattern in small biochemical systems, *J. R. Soc. Interface* **20**, 20230276 (2023).
- [68] U. Seifert, Stochastic thermodynamics, fluctuation theorems and molecular machines, *Rep. Prog. Phys.* **75**, 126001 (2012).
- [69] A. T. Winfree, *The Geometry of Biological Time* (Springer, New York, 2001), Vol. 12.
- [70] Y. Kuramoto, *Chemical Oscillations, Waves, and Turbulence* (Springer, Berlin, Heidelberg, 2003).
- [71] Y. Song, J. O. Park, L. Tanner, Y. Nagano, J. D. Rabinowitz, and S. Y. Shvartsman, Energy budget of *Drosophila* embryogenesis, *Curr. Biol.* **29**, R566 (2019).
- [72] X. Yang, M. Heinemann, J. Howard, G. Huber, S. Iyer-Biswas, G. Le Treut, M. Lynch, K. L. Montooth, D. J. Needleman, S. Pigolotti, *et al.*, Physical bioenergetics: Energy fluxes, budgets, and constraints in cells, *Proc. Natl. Acad. Sci. USA* **118**, e2026786118 (2021).
- [73] I. Di Terlizzi, M. Gironella, D. Herraiz-Aguilar, T. Betz, F. Monroy, M. Baiesi, and F. Ritort, Variance sum rule for entropy production, *Science* **383**, 971 (2024).
- [74] Z. Padamsey and N. L. Rochefort, Paying the Brain's energy bill, *Curr. Opin. Neurobiol.* **78**, 102668 (2023).
- [75] S. D. Jamadar, A. Behler, H. Deery, and M. Breakspear, The metabolic costs of cognition, *Trends Cognit. Sci.* **29**, 541 (2025).
- [76] M. B. Elowitz and S. Leibler, A synthetic oscillatory network of transcriptional regulators, *Nature (London)* **403**, 335 (2000).
- [77] J. Stricker, S. Cookson, M. R. Bennett, W. H. Mather, L. S. Tsimring, and J. Hasty, A fast, robust and tunable synthetic gene oscillator, *Nature (London)* **456**, 516 (2008).
- [78] L. Potvin-Trottier, N. D. Lord, G. Vinnicombe, and J. Paulsson, Synchronous long-term oscillations in a synthetic gene circuit, *Nature (London)* **538**, 514 (2016).