

Why life is hot

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(Dated: February 13, 2026)

The process of evolution by natural selection leads to phenotypes of increasing fitness. For cellular chemical reaction networks, this means optimising a variety of fitness functions such as robustness, precision, or sensitivity to external stimuli. We argue that these diverse goals can be achieved by a versatile, generic mechanism: coupling chemical reaction networks to reservoirs that are strongly out of equilibrium. Using theory and numerics we show that this mechanism of optimization comes at the price of significant heat dissipation. We compute the heat flux caused by kinetic proof-reading in *Escherichia coli* and show that it constitutes a significant fraction of the total heat flux experimentally measured in this model organism. We then demonstrate that the degree of optimality achievable saturates, and that Nature appears to operate near saturation despite high energetic costs. We conclude that ‘life is hot’ largely because of the need for a versatile mechanism to optimise a variety of fitness functions.

I. INTRODUCTION

Two features of life are typically taken as self evident: first that it is a non-equilibrium phenomenon, and second that it ubiquitously generates heat. That ‘life is non-equilibrium’ is frequently discussed, especially in the physics literature [1], because non-equilibrium statistical mechanics continues to pose formidable challenges. In contrast, that life dissipates heat is considered so banal that it seldom draws comments except in textbooks.

Interestingly, the most common textbook explanation of why life must dissipate heat, e.g., as given in successive editions of a well-known university text [2], is incorrect. Alberts et al. explain that the cellular metabolism generates ‘order’, which lowers entropy. (Schrödinger coined the term ‘negentropy’ for this effect [3].) To satisfy the Second Law of Thermodynamics, enough heat must be exported from the cell to raise the entropy of the environment to give a net positive entropy change. However, numerical estimates of the total entropy reduction due to various ordering processes within a cell (polymerisation, compartmentalisation, etc.) show that this argument underpredicts the heat production by around two orders of magnitude [4]. Cells reject much more heat than is needed to compensate for their ‘negentropy’. Why, then, does life ubiquitously produce heat?

Before we give an answer to this question, we need to set some preliminaries. First, metabolic rates are almost universal across a large range of organisms from all domains. They stay within a narrow band of $1\text{--}100\text{ W kg}^{-1}$

for organisms spanning 20 orders of magnitude of body mass [5]. Not all of the energy that an organism takes up from its environment is converted into heat – clearly, living organisms need to perform chemical and mechanical work to survive – but the heat flux amounts to 60% to 90% of the energy balance [6–8]. From a physicist’s perspective this observation contains two aspects that require an explanation: the quasi-universal behaviour across all domains and the apparent ‘waste’ of energy as heat.

Second, note that only a small fraction of all organisms are endotherms (i.e. regulate their own body temperatures). Most organisms dissipate heat without increasing their temperature. The dissipated heat is taken up by the surrounding medium which acts as a heat bath. Regulation of the body temperature is vital for some animals, but it cannot be the general explanation of heat dissipation in all life-forms.

Finally, note that ‘non-equilibrium’ does not necessitate ‘hot’. Indeed, the extraction of $-\Delta G > 0$ useful work from a chemical reaction at constant temperature and pressure may involve *absorbing* heat from its environment. To see this, recall that under quasi-static conditions $Q = T\Delta S = \Delta H - \Delta G$ where (Q, T, S, H) are the absorbed heat, temperature, entropy, and enthalpy respectively. If $\Delta S > 0$ then $Q > 0$, the quasi-static reaction is endothermic; this is the case for example in the quasi-static oxidation of carbon or glucose under standard conditions [9–11].

We will demonstrate that, instead, the cause for heat dissipation lies in the evolutionary pressure to optimize chemical reaction networks for a variety of functions. Sometimes organisms need to be precise (e.g. when translating mRNA into proteins); sometimes they need

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to produce superstructures with tightly-defined properties (e.g. long actin filaments with a narrow length distribution); and sometimes they need to respond at speed to external stimuli (e.g. heat shock). We show that adding phosphorylation-dephosphorylation cycles to chemical reaction networks is a versatile way to optimise them for a variety of fitness functions, but necessarily dissipates a significant amount of heat.

The article is structured as follows: First we discuss the role of cycles in the optimization of chemical reaction networks. Then we compute the heat flux in two well-known biochemical processes. We then compare our prediction of the heat dissipated by kinetic proofreading in *Escherichia coli* to results of calorimetry experiments and conclude that proofreading causes a significant fraction of the total heat flux. Further, we show that the degree of optimality saturates, and that Nature appears to operate near saturation. Finally, we suggest an explanation for the observed quasi-universality of metabolic rates.

A. Phosphorylation-dephosphorylation cycles optimize fitness

We begin our discussion with an example of a biochemical optimization problem that is solved by means of a driven reaction cycle. To enable the proper functioning of the cytoskeleton, actin filaments need to have a well-defined length [12]. If actin polymerization proceeded under thermodynamic equilibrium conditions, there would be a large dispersity in filament lengths. In order to reduce the length dispersity, cells couple the polymerization process to the dephosphorylation of ATP as sketched in Fig. 1 [13].

There are two types of ‘monomeric’ actin, ATP bound (yellow spheres) and ADP bound (orange spheres). The ends of the filament have different structures (indicated by plus and minus signs). The rates of attachment and detachment differ between the ends as well as between the types of monomers (in the sketch the magnitude of the rate is indicated by the thickness of the arrow). If the dephosphorylation step were missing, none of these details would matter. The length distribution would always be Poissonian¹. The dephosphorylation step, which changes the properties of the monomer units while they are part of the polymer, is strongly out of equilibrium and therefore affects the nature of the length distribution. In the so-called ‘treadmilling regime’ the distribution becomes a convolution of a Poissonian with a rather

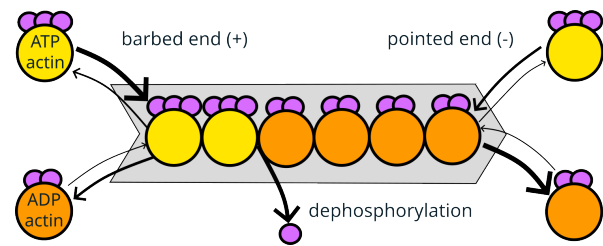


FIG. 1. Sketch of actin polymerization. Yellow spheres denote ATP-actin, orange spheres denote ADP-actin, the individual purple sphere denotes inorganic phosphate. The thickness of the arrows indicates the magnitude of the reaction rate.

narrow Gaussian [13–17]. Thus the phosphorylation-dephosphorylation cycle reduces the dispersity in filament lengths.

Cycles of this form appear ubiquitously in biochemistry. Further examples are:

- In protein synthesis the error is reduced with respect to its equilibrium value by means of the Hopfield kinetic proofreading cycle [18–20]. Here, the quantity which is optimized is the ratio between two currents. (We discuss the details in sec. II C.)
- When heat shock proteins bind to the proteins that they refold, they show ‘ultra-affinity’. In this case a dephosphorylation cycle is used to enhance a binding affinity over its equilibrium value [21, 22].
- In chemotaxis the sensitivity is optimized by a reaction cycle that is equivalent in structure to the Hopfield proofreading cycle [23].

To see why such cycles are useful, we consider the master equations for a network of chemical reactions. We denote by $c_i(t)$ the concentration of chemical species i and by k_{ij} and the reaction rate between species i and j . The concentrations evolve as

$$\frac{dc_i(t)}{dt} = \sum_j (k_{ij}c_j(t) - k_{ji}c_i(t)) = \sum_i J_{ij} \quad , \quad (1)$$

where we have defined the currents $J_{ij}(t) \equiv k_{ij}c_j(t) - k_{ji}c_i(t)$. We are, in particular, interested in the steady state solutions of eq. (1), $c_i(t) = \text{const} \equiv c_i^s$.

Consider now how we may optimize the steady state of the network with respect to some fitness function. To give a few examples, this function could be the dispersity Δc_i^s in one of the concentrations (as in the case of the actin filaments), the speed with which a certain particle species is produced, the average time two species remain in a complex, or the response of a concentration to a small change in a current, $\partial c_i^s / \partial J_{jk}^s$.

If the network is tree-like, i.e. if it does not contain any loops, the steady-state concentration ratios c_i^s / c_j^s are given by the detailed balance condition $J_{ij}^s = k_{ij}c_j^s - k_{ji}c_i^s = 0$ for all i, j [24]. Thus the absence of loops

¹ Living polymerization of a single particle species in equilibrium produces a Poissonian length distribution. The combination of several different particle species and different attachment/detachment rates at the ends of the polymer, if they occur independently, result in a convolution of several Poissonians, which is again a Poissonian.

significantly limits the set of functions that can be optimized. To change the concentration ratios in a tree-like network, one must either alter rates k_{ij} or add species and therefore reactions to the network. Both mechanisms occur in living organisms, e.g., via point mutations to extant proteins or the emergence of (say) a novel inhibitor. However, either mechanism requires a one-off, specific chemical innovation as each new challenge arises; neither is a suitable ‘design principle’ for a generic solution.

To design a generic solution for optimising networks for multiple fitness functions without changing the chemistry of the constituents, we need to include cycles. One way of adding a cycle to a network is to fix the concentrations of two or more of the species by attaching particle reservoirs. The concentrations in the reservoirs need to be maintained by means of a second chemical reaction network, hence the presence of reservoirs implies the presence of cycles.

In a network with cycles, the steady-state currents $J_{i,j}^s$ can be different from zero. It is now possible to tune fitness functions which depend on the concentrations, the currents and their dispersities. In particular, the sensitivities $\partial \ln c_i^s / \partial \ln J_{j,k}^s$ can be optimized. The extreme case of optimization by means of a cycle concerns the so-called ‘futile’ cycles in which a chemical species is disassembled into its building blocks only to be reassembled again without any obvious purpose. However, if a ‘futile’ cycle is attached to a reaction network, the steady state concentrations are shifted away from their equilibrium values and thus the sensitivity of the network to the boundary conditions is altered. As has been previously pointed out, the cycle is not at all futile if it is placed at the right node to tune the network’s response to variations in currents or concentrations [25–27].

In living organisms, perhaps the most versatile cycles are those involving the dephosphorylation of ‘energy currency’ metabolites such as ATP, which are chemiostat-² at concentrations very far from their equilibrium values [29]. According to biology textbooks, ATP is used for the following purposes: to enable reactions that are energetically unfavourable, to transport substances across membranes, and to do chemical or mechanical work [2]. We argue that ATP has a fourth and at least equally important task: to optimize the emergent properties of chemical reaction networks by driving them strongly out of equilibrium.

A large variety of fitness functions can be tuned by means of NTP-ases (i.e. enzymes that catalyse the dephosphorylation of nucleoside-triphosphates such as ATP) which couple phosphorylation-dephosphorylation cycles into chemical reactions. However, this advantage comes at a cost that is manifested as a ubiquitous characteristic of life: a significant amount of heat is produced.

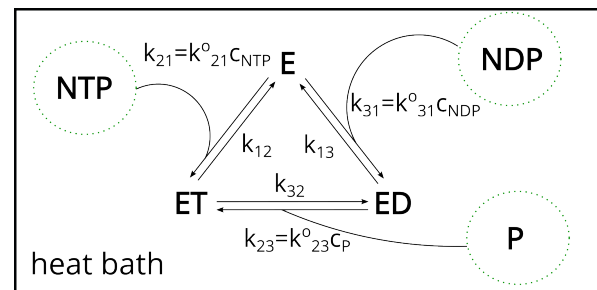


FIG. 2. Reaction network coupled to a heat bath, which fixes the temperature T , and to three particle reservoirs (indicated by dashed circles), which control the concentrations of the species NTP, NDP and P.

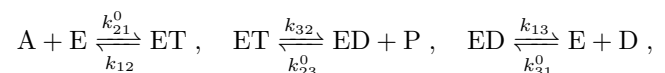
II. RESULTS

A. Dissipation in Chemiostatted Biochemical Networks

To estimate the amount of heat that is produced, consider a chemical reaction network which is attached to three particle reservoirs and embedded in a heat bath at a temperature T , Fig. 2.³ The system cycles through the states E, ET and ED transporting molecules from the NTP-reservoir to the NDP- and P-reservoirs, i.e. the species E acts as a catalyst for the reaction



Species E takes part in three reactions



where k_{ij} are first order rate constants and k_{ij}^0 are second order rate constants, i.e. the corresponding rates are given by products with concentrations such as $k_{21}^0 c_{\text{NTP}} =: k_{21}$. The cyclic process has to be possible, *inter alia*, in equilibrium. Hence the reaction rates need to fulfill the condition

$$\frac{k_{21}^0 k_{32} k_{13}}{k_{12} k_{23} k_{31}^0} = \frac{c_{\text{NDP}}^{\text{eq}} c_{\text{P}}^{\text{eq}}}{c_{\text{NTP}}^{\text{eq}}}, \quad (3)$$

where c_i^{eq} is the concentration of species i in equilibrium.⁴

² In the language of stochastic thermodynamics, fixing concentrations by means of reservoirs is called ‘chemiostatting’ [28].

³ In real biochemistry, such a network will be part of a larger metabolic pathway. We analyze this as a self-contained structure only as a minimal model to estimate heat production in driven reactions. The letters NTP do not refer to a specific species of molecule, but are a place holder for ATP, GTP, other nucleoside triphosphates or complexes formed with any of these molecules. We will specify details later in the examples.

⁴ To see this, consider the special case in which the concentrations in the reservoirs are fixed to the equilibrium values of Reaction (2). Then the product of the ratios of forward to backward rates $(k_{21}^0 c_{\text{NTP}}^{\text{eq}} / k_{12}) \cdot (k_{32} / (k_{23}^0 c_{\text{P}}^{\text{eq}})) \cdot (k_{13} / (k_{31}^0 c_{\text{NDP}}^{\text{eq}}))$ has to equal 1.

If we set the concentrations in the NTP, NDP and P reservoirs to values other than the equilibrium concentrations, the system runs in a non-equilibrium steady state (NESS). The change of free energy ΔG in the NESS is determined solely by the properties of NTP, NDP and P

$$\frac{\Delta G}{k_B T} = \ln \left(\frac{Q}{K} \right), \quad (4)$$

where k_B is Boltzmann's constant, $Q = c_{NDP} c_P / c_{NTP}$ is the reaction quotient, c_{NDP} , c_P and c_{NTP} denote the reservoir concentrations, and $K = c_{NDP}^{eq} c_P^{eq} / c_{NTP}^{eq}$ is the equilibrium constant for Reaction (2). (Note that we use two similar looking letters: Q for the reaction quotient and Q for heat.)

In the NESS heat is dissipated at a rate [27, 30]

$$\frac{dQ}{dt} = k^s c_E^{tot} \Delta G, \quad (5)$$

where $c_E^{tot} = (c_E^s + c_{ET}^s + c_{ED}^s)$ is the total concentration of species E , and the rate k^s is defined via the steady state current⁵

$$J^s \equiv \sum_{i,j \neq i} (k_{ij} c_i^s - k_{ji} c_j^s) \equiv k^s c_E^{tot}. \quad (6)$$

Depending on the specific ‘energy currency’, the temperature, pH and other conditions, $K \simeq 10^5$ – 10^6 M [31–33]. In cells, the concentration of energy sources containing ATP or GTP as well as the concentration of inorganic phosphate is in the millimolar range, while the concentrations of hydrolysed forms (ADP etc.) is typically in the micromolar range [34]. Hence, in a biological organism, the reaction quotient $Q = c_{NDP} c_P / c_{NTP}$ is of the order 10^{-5} – 10^{-6} M, which corresponds to $K/Q \sim 10^9$ – 10^{12} , or equivalently $\Delta G \simeq 21$ – $28 k_B T \simeq 50$ – 70 kJ mol⁻¹. These are very large numbers. After providing some numerical estimates of heat production in biochemical reactions, we will propose an explanation of why natural selection has ‘tuned’ K/Q to such large values.

B. Example (1): actin treadmilling

To estimate the magnitude of heat dissipation, Eq. (5), for actual biochemistry, we consider first actin treadmilling (Fig. 3) [13, 14, 16, 17, 35]. The chain of bound F-actin subunits corresponds to species E . First, G-actin monomers are activated by binding to ATP (yellow spheres in Fig. 3). Activated monomers are then added to the ‘barbed’ end of the filament ($E \rightleftharpoons ET$). While they are part of the filament they are hydrolyzed ($ET \rightleftharpoons ED$),

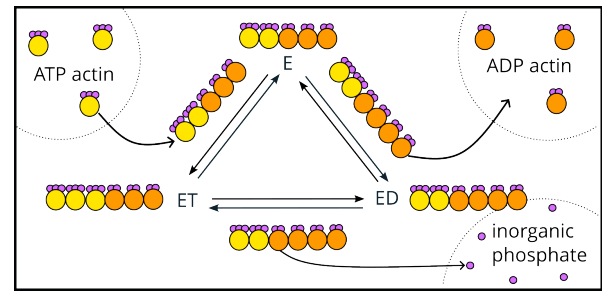


FIG. 3. Actin treadmilling. Yellow spheres with three purple spheres denote ATP-actin, orange spheres with two purple spheres denote ADP-actin, individual purple spheres denote inorganic phosphate.

and finally they drop off at the ‘pointed’ end ($ED \rightleftharpoons E$). As the hydrolysis step is strongly irreversible, the system enters the NESS known as ‘treadmilling’ [13].

Steady state fluxes in this process are on the order of 10 subunits per second added to/removed from a filament [14, 15, 36]. Hence we expect the heat production rate of a single actin filament to be of the order

$$\left(\frac{dQ}{dt} \right)_{\text{filament}} \simeq \frac{10 \text{ s}^{-1} \times 60 \text{ kJ mol}^{-1}}{6 \times 10^{23} \text{ mol}^{-1}} \simeq 10^{-18} \text{ W}.$$

There are considerable spatiotemporal variations in actin filament concentration in cells. It is particularly high, ~ 1 mM, in the lamellipodia of migrating fibroblasts [12, 37]. Assuming that the filaments have a length of order 10^2 subunits [38], we expect to see a heat production of

$$\left(\frac{dQ}{dt} \right)_{\text{lamellipodia}} \simeq 5 \text{ W kg}^{-1}, \quad (7)$$

which lies within the range of measured values for the metabolic rate in a diversity of organisms that we referred to in the introduction [5, 39]. More detailed experimental testing of our estimate could perhaps come from calorimetric measurements of fragments shed from fish keratocytes that are essentially pieces of ‘pure cytoskeleton’ engaged in lamellopodia-like motion [40].

C. Example (2): kinetic proofreading

An even more ubiquitous example of a chemiostatted cycle is the kinetic proofreading cycle (Fig. 4), which regulates the error in translating mRNA to proteins in all organisms [18, 19]. To make numerical estimates, we consider proofreading in *E. coli*. In this case the species E from Fig. 2 corresponds to the ribosome and the species NTP to the elongation factor complex between aminoacyl-tRNA and GTP. In the stationary (non-growing) phase, *E. coli* contains between 5000 and 10000 ribosomes per cell (i.e. about 10^{-20} mol). In the exponential growth phase there are up to 55000 ribosomes

⁵ The rate k^s is determined by the individual reaction rates and the concentrations in the reservoirs. We will return to it in subsection IID, and we give a detailed expression in Eq. (9). For a justification of Eq. (5), see sec. IV A.

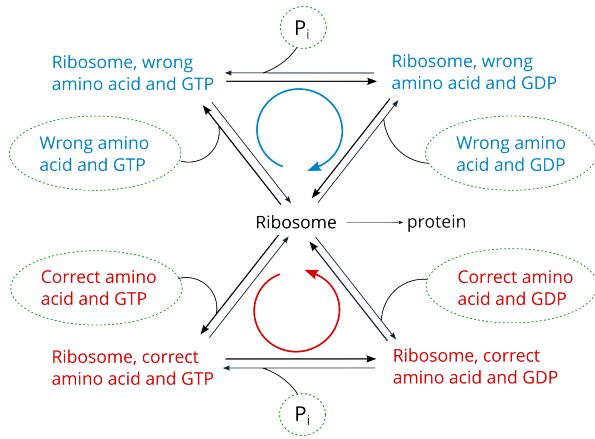


FIG. 4. Sketch of the kinetic proofreading cycle [18, 19]. The reaction network consists of two cycles of the form shown in Fig. 2, one for the correct amino acid (red) and one for the wrong amino acid (blue). The fitness function that is optimized is the ratio of the ‘wrong current’ to the ‘correct current’ at the node of the ribosome.

(i.e. about 10^{-19} mol) [41]. The step $ET \rightleftharpoons ED$ consists of GTP activation and hydrolysis. Experimentally-determined rates for the combination of these processes vary between $k_{32} \simeq 25 \text{ s}^{-1}$ [42] and 54 s^{-1} [43]. We know of no measured value of k_{13} , and so will follow the strategy of Zuckerman [44, 45] and use the measured value of k_{12} as an estimate, $k_{13} \simeq k_{12} \simeq 100 \text{ s}^{-1}$ [43]. These constants give us $k^s \simeq 20\text{--}35 \text{ s}^{-1}$, which in turn yields a heat production rate per bacterium of

$$\left(\frac{dQ}{dt}\right)_{\text{kinpr}} \simeq \begin{cases} 0.2 \text{ pW} & (\text{exponential phase}), \\ 0.02 \text{ pW} & (\text{stationary phase}). \end{cases} \quad (8)$$

As an *E. coli* bacterium has a mass of about 1 pg [46], these values correspond to $(dQ/dt)_{\text{kinpr}} \simeq 200 \text{ W kg}^{-1}$ in the exponential phase and $(dQ/dt)_{\text{kinpr}} \simeq 20 \text{ W kg}^{-1}$ in the stationary phase, which again lies in the range of measured values given in ref. [5, 39].

The heat dissipation rate of actively growing *E. coli* has been measured directly by calorimetry. In the exponential phase, *E. coli* in various nutrient broths dissipate $\simeq 1\text{--}4 \text{ pW}$ per cell [47], so that kinetic proofreading, eq. (8), is apparently responsible for a significant fraction of this dissipation. This seems reasonable, given that protein synthesis, of which kinetic proofreading is a key component, has been estimated to consume up to two-thirds of the energy budget of an active growing *E. coli* cell [48].

It is instructive to inquire into the origins of the remainder of the $\gtrsim 1 \text{ pW}$ heat dissipation in actively growing *E. coli*. To do so, we consider how much heat is produced by the process of chemiostatting, i.e. of maintaining the ATP reservoir. For this we turn to two highly-curated models of the metabolism for *E. coli* developed for use within the framework of flux-balance analysis (FBA) [49] (see Materials and Methods, sec. IV).

For fast-growing *E. coli* (doubling time of order 40–50 min) on a glucose minimal medium under aerobic conditions, we determined the total ATP maintenance demand is 60–80 mmol per gram dry weight (gDW) of biomass, of which 6–10% is for so-called non-growth-associated maintenance (NGAM). Assuming all of this ATP is subsequently hydrolysed in ‘futile’ cycles, this corresponds to a heat production rate of 0.3–0.4 pW per bacterium.

A similar conclusion is reached if the NGAM component, contributing 0.02–0.04 pW per bacterium, is interpreted as predicting the heat production for maintaining the ATP reservoir concentrations in the stationary phase.⁶

We conclude that the heat dissipated due to kinetic proofreading, which is the sum of the heat dissipated by the NESS in the proofreading cycle and by refilling the reservoirs, makes a significant or even dominant contribution to the overall heat production in both exponential growth and stationary phase.

D. Steady-State Current

Finally, we answer the question why Nature maintains the reservoir concentrations of nucleoside triphosphates so far from their equilibrium values, i.e. $-\Delta G$ at such a high value. To do so, we return to the steady state current, Eq. (6). The rate k^s is the difference between the product of the ‘anticlockwise rates’, $k_{21}k_{32}k_{13}$, and the product of the ‘clockwise rates’, $k_{12}k_{23}k_{31}$

$$k^s = \frac{k_{21}k_{32}k_{13} - k_{12}k_{23}k_{31}}{d}, \quad (9)$$

normalized by

$$d = k_{21}k_{32} + k_{32}k_{13} + k_{13}k_{21} + k_{31}k_{23} + k_{23}k_{12} + k_{12}k_{31} + k_{13}k_{12} + k_{21}k_{23} + k_{32}k_{31},$$

where we have set $k_{21} = k_{21}^0 c_{\text{NTP}}$, $k_{23} = k_{23}^0 c_{\text{P}}$, and $k_{31} = k_{31}^0 c_{\text{NDP}}$.

If the flux is predominantly anticlockwise ($k_{21}k_{32}k_{13} \gg k_{12}k_{23}k_{31}$), re-phosphorylation is negligible ($k_{23}^0 c_{\text{P}} \ll k_{21}^0 c_{\text{NTP}}$) and re-attachment of the hydrolysed complex is also small ($k_{31}^0 c_{\text{NDP}} \ll k_{21}^0 c_{\text{NTP}}$), the steady state current approaches

$$J_{\text{lim}}^s = \frac{k_{32}k_{13}}{k_{32} + k_{13} + k_{23}^0 c_{\text{P}}} c_{\text{E}}^{\text{tot}}, \quad (10)$$

⁶ These estimates are admittedly rather crude, since we use the ATP maintenance demand as a proxy for other currency metabolites like GTP and we neglect for example the existence of overflow metabolism and other ‘sinks’ for the ATP. In principle, the calculation could be refined to include these effects. However, precision to this level is beyond the scope of the present work.

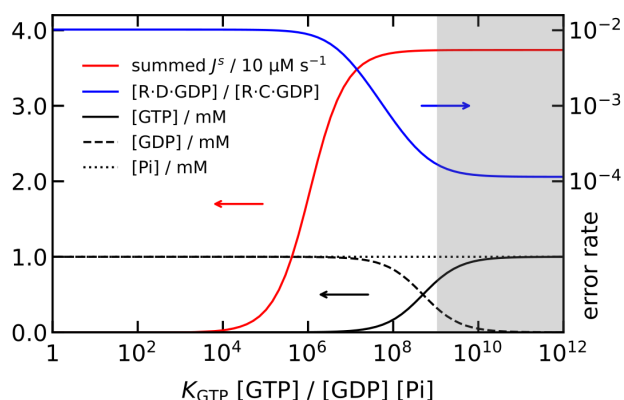


FIG. 5. Kinetic proof reading model: summed cycle flux (red) and error rate (blue), expressed as a function of K/Q for the GTP hydrolysis equilibrium. (Square brackets indicate concentrations.) The error rates saturates at $K/Q \simeq 10^{10}$. The shaded area indicates the range of K/Q observed in living organisms [34].

For the calculation, the GTP : GDP ratio is varied, constraining $[GTP] + [GDP]$ and $[Pi]$ to 1 mM. Elongation factor complex concentrations are 0.1 mM for both ‘C’ (correct) and ‘D’ (wrong) amino acid types. A copy number of 5000 ribosomes is assumed in a cell volume $2 \mu\text{m}^3$ (concentration $\simeq 4.2 \mu\text{M}$). Detachment rates of the complexes are enhanced by a factor $f = 100$ for the ‘wrong’ amino acid type. For the saturated total cycle flux $J^s/[R]_{\text{tot}} = k^s \simeq 10 \text{ s}^{-1}$, similar to the range quoted in the main text above (8). (For more details see Materials and Methods, sec. IV.)

i.e. it saturates at a value that is independent of the reservoir concentrations c_{NTP} and c_{NDP} . As a consequence, chemiostatting the reservoir concentrations even further out of equilibrium would not change the values of any fitness functions anymore. This saturation effect has been observed in the translation error in kinetic proof reading, which cannot be reduced beyond a certain limit per proof-reading cycle [25, 50], and in ultra-sensitivity [23]. Generally, when using a cycle of the type shown in Fig. 2 in order to optimize a fitness function, there is a reaction quotient, beyond which nothing is gained anymore by converting even more chemical energy into heat.

To explore this conclusion quantitatively, we implemented a chemiostatted version of a previously-described proofreading model [44, 51], which includes a cycle for the attachment of the correct amino acid to the growing polypeptide chain, as well as a prototypical cycle for the mistaken attachment of a ‘wrong’ amino acid; in the latter the elongation factor complex detachment rates are enhanced. We used rate coefficients and concentrations representative of the situation described above (see sec. IV for details).

Figure 5 shows the cycle flux summed over both cycles and the error rate (i.e. the rate of attachment of ‘wrong’ amino acid versus the correct amino acid) as a function of the disequilibrium measure K/Q for $GTP \rightleftharpoons GDP + Pi$. The shaded area in Fig. 5 indicates the range of K/Q

values observed in living organisms. The proofreading effect is clearly seen in the dramatic drop off in the error rate (blue line) for $K/Q \gtrsim 10^7$. Interestingly, the summed cycle flux (red line), which is dominated by the attachment of correct amino acids, appears to saturate earlier than the proofreading effect. This can be traced to the delayed saturation of the ‘wrong’ attachment cycle. Optimal proofreading requires $K/Q \gtrsim 10^{10}$. Interestingly, the shaded region begins at around this value. This simple model therefore explains the physiological requirement for such a large value of ΔG for the fueling currency metabolite (GTP), with the accompanying large heat production arising from its continual consumption.

Before we conclude, we briefly return to the question why the metabolic rates of a large variety of organisms are almost universal. We recall that the concentration of inorganic phosphate is usually in the micromolar range, thus the term $k_{23}^0 c_P$ in J_{lim}^s (eq. (10)) is negligible and the rate k^s approximately equals

$$k_{\text{lim}}^s \simeq \frac{k_{32}k_{13}}{k_{32} + k_{13}}, \quad (11)$$

which is the harmonic mean of the rate of dephosphorylation and the rate by which the hydrolysed complex drops off the enzyme. These rates do vary between different nucleoside-triphosphatases, but not by many orders of magnitude. Hence it stands to reason that the observed values for the heat, eq. 5, stay in a limited range.

III. SUMMARY AND CONCLUSION

We have presented three hypotheses:

1. To couple chemiostatted phosphorylation-dephosphorylation cycles into chemical reaction networks is a versatile mechanism to optimize fitness functions.
2. Most of the heat produced by biological organisms is a necessary byproduct of this mechanism.
3. Metabolic rates are quasi-universal across all domains, because the mechanism of optimization is operated at saturation.

We have analyzed the heat dissipation caused by kinetic proofreading in *E. coli* and we have shown that it constitutes a significant part of the heat measured in calorimetric experiments on this model organism. Further we have shown that the effect of the optimization mechanism saturates and that Nature operates at saturation.

In his 2025 Nobel Prize lecture Hopfield pointed out: “‘Free energy’ is the key. High-energy molecules like glucose or adenosine triphosphate are expensive for a cell to make, and a biochemical process using unexpectedly large numbers of high-energy molecules in a simple process must be paying that cost for a purpose.” [52]

This argument can be taken further. Most of the energy contained in the currency metabolites is not transformed into chemical work, but dissipated as heat into an organism's environment. This might seem wasteful at first glance, but it provides living organisms with a flexible tool to optimize their fitness. That Nature has driven this mechanism into saturation means that living organisms do not make a compromise between a thrifty use of resources and a moderate improvement of chemical reaction networks. Rather, they take up all the energy needed to run the mechanism optimally even if most of the energy is 'wasted' as heat. This indicates that from an evolutionary perspective, being able to adapt flexibly to various fitness functions is advantageous over being economical with energy consumption. Life is therefore hot because it is versatile *and* adaptive.

IV. MATERIALS AND METHODS

A. Computation of the heat dissipation rate

In the NESS of the reaction network shown in Fig. 2 entropy is produced at a rate [27, 30]

$$\begin{aligned} \frac{dS^{\text{sys}}(t)}{dt} &= \frac{k_B}{2} \sum_{i,j \neq i} (k_{ij} c_i^s - k_{ji} c_j^s) \ln \left(\frac{k_{ij} c_j^s}{k_{ji} c_i^s} \right) \\ &= \frac{1}{T} J^s \Delta G, \end{aligned} \quad (12)$$

with $i, j \in \{E, ET, ED\}$. Here we have introduced the steady state current $J^s \equiv \sum_{i,j \neq i} (k_{ij} c_i^s - k_{ji} c_j^s)$ and we have used the notation dS^{sys}/dt for the change in entropy that is not due to heat exchange.⁷

If the particle reservoirs are kept strictly at constant concentrations, all of the produced entropy enters the heat bath. Otherwise some of the entropy enters the particle reservoirs and would not be detected in a calorimetry experiment. For a discussion of this issue see reference [28], and for an exhaustive analysis of the stochastic thermodynamics of chemical reaction networks with particle reservoirs see reference [54]. As we are interested in estimating orders of magnitude, we focus on the idealized case of perfect chemiostatting. We obtain for the rate of heat production

$$\frac{dQ}{dt} = T \frac{dS^{\text{sys}}(t)}{dt} = J^s \Delta G. \quad (13)$$

B. Kinetic proof reading model

To generate the results shown in Fig. 5, we implemented the following model, which hews closely to that

described in Refs. [44, 45]. The model comprises quasi-chemical reactions that represent: elongation factor (EF) complex formation (association), $C + \text{GTP} \rightleftharpoons C \cdot \text{GTP}$; ribosomal binding $R + C \cdot \text{GTP} \rightleftharpoons R \cdot C \cdot \text{GTP}$; hydrolysis, $R \cdot C \cdot \text{GTP} \rightleftharpoons R \cdot C \cdot \text{GDP} + \text{Pi}$; detachment, $R \cdot C \cdot \text{GDP} \rightleftharpoons R + C \cdot \text{GDP}$; and dissociation, $C \cdot \text{GDP} \rightleftharpoons C + \text{GDP}$. All reactions are assumed reversible, though out of equilibrium some are strongly driven in the forward direction. Rate coefficients are, respectively, in the forward and back directions, $g'_t = k_{\text{on}} \equiv 10^8 \text{ M}^{-1} \text{ s}^{-1}$, $g_t = k_{\text{off}} \equiv 100 \text{ s}^{-1}$; $k' = k_{\text{on}}$, $k = k_{\text{off}}$; $m' = 0.1 k_{\text{off}}$, for m , see below; $l = k_{\text{off}}$, $l' = 0.01 k_{\text{on}}$; $g_d = 10 k_{\text{off}}$, $g'_d = k_{\text{on}}$. A so-called 'cycle constraint' fixes the value of m in the hydrolysis step according to $m'/m = K_{\text{GTP}}(l'/l)(k/k')(g_t/g'_t)(g'_d/g_d)$, where K_{GTP} is the equilibrium constant for the hydrolysis equilibrium $\text{GTP} \rightleftharpoons \text{GDP} + \text{Pi}$; we take $K_{\text{GTP}} = 5 \times 10^5 \text{ M}$ so that $m = 0.02 \text{ M}^{-1} \text{ s}^{-1}$. The cycle constraint ensures that the cycle flux vanishes when the concentrations of GTP, GDP and inorganic phosphate (Pi) are at their equilibrium values. We do not explicitly represent polypeptide elongation in the model since it has a very small rate compared to the cycle reactions; rather this step is assumed proportional to the steady-state concentration of the hydrolysed bound EF complex $[R \cdot C \cdot \text{GDP}]$. We also omit direct hydrolysis of GTP and $C \cdot \text{GTP}$ since the rates can be assumed negligible under the relevant conditions. Finally, as in Ref. [44], a parallel set of reactions with 'D' representing the attachment of a 'wrong' amino acid are also included, with detachment rates k and l increased by a factor $f = 100$ compared to 'C'; the translation error rate is then defined as the ratio $[R \cdot D \cdot \text{GDP}]/[R \cdot C \cdot \text{GDP}]$. We assume the currency metabolites are chemiostatted as $[\text{GTP}] = [\text{G}][\text{Pi}]/([\text{Pi}] + Q_{\text{GTP}})$ and $[\text{GDP}] = [\text{G}]Q_{\text{GTP}}/([\text{Pi}] + Q_{\text{GTP}})$, where $Q_{\text{GTP}} = [\text{GDP}][\text{Pi}]/[\text{GTP}]$ is the reaction quotient for the notional GTP hydrolysis equilibrium and $[\text{G}] = [\text{GTP}] + [\text{GDP}]$ is the held-constant phosphorylation-state agnostic total concentration of the currency metabolite. To obtain the results shown in Fig. 5, we set the total individual concentrations of C and D at 0.1 mM, calculate the total ribosomal concentration assuming a copy number of 5000 ribosomes in a volume $2 \mu\text{m}^3$, take $[\text{G}] = [\text{Pi}] = 1 \text{ mM}$ as typical physiological values, and solve for the non-equilibrium steady state (NESS) as a function of the degree of disequilibrium by varying the reaction quotient Q_{GTP} between $5 \times 10^{-7} \text{ M}$ and the equilibrium upper bound $K_{\text{GTP}} = 5 \times 10^5 \text{ M}$. Different choices here all result in similar phenomenology. We use the BASICO interface [55] to the COPASI simulation platform [56] to perform the actual calculations. A Python script which implements the model and generates Fig. 5 is available on request.

⁷ The biochemical literature often follows Prigogine and uses notation $d_i S(t)/dt$ for this quantity [53].

C. Flux-balance analysis

We used two recent genome-scale metabolic reconstructions, iJO1366 [57] and iML1515 [58], which represent comprehensive models of the metabolic capabilities of *E. coli*, to examine the ATP maintenance demands. We adopted the default growth medium which comprises glucose as the sole carbon/energy source; ammonium, inorganic phosphate and sulfate ions to satisfy the elemental demands for N, P, and S in the biomass; and sundry trace nutrients and minerals unimportant for present purposes. Under aerobic conditions, and limiting the glucose uptake rate to $10 \text{ mmol gDW}^{-1} \text{ hr}^{-1}$, we optimise for the flux to biomass using flux-balance analysis (FBA) [49]. This yields growth rates of $0.98\text{--}0.88 \text{ hr}^{-1}$, where here and in the main text the figure for iJO1366 is given first. We then interrogate the models to determine the grow-associated ATP maintenance (GAM) demand incorporated in the flux to biomass, and the non-growth associated ATP maintenance (NGAM) demand associated to the reaction $\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{H}^+ + \text{P}_i$. Combining these gives the result quoted in the main text.

To do the heat production calculation we assume a reaction enthalpy $\Delta H \simeq -30.5 \text{ kJ mol}^{-1}$ for ATP hydrolysis, take the dry mass of an *E. coli* bacterium as $\simeq 0.43 \text{ pg}$, and divide by the doubling time to get the quoted heat production rates. The models were downloaded from the BiGG Models database [59], and used as given. FBA calculations were performed using the COBRApy platform [60]. The Python scripts which perform the calculations are available on request.

ACKNOWLEDGMENTS

WCKP thanks the Isaac Newton Institute for Mathematical Sciences, Cambridge, for supporting participation in the 2023 ‘New statistical physics in living matter’, where the idea for this work was first conceived. TS thanks the Higgs Centre for Theoretical Physics, The University of Edinburgh, for part funding a sabbatical visit to Edinburgh, where the research was initiated and substantially completed. We thank Anja Seegebrecht, Thorsten Friedrich, Thorsten Hugel, Daan Frenkel, Andreas Härtel and Stefan Miller for feedback on the manuscript.

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