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Perspective

Do We Understand the Mechanisms Used by Biological Systems to Correct Their Errors?

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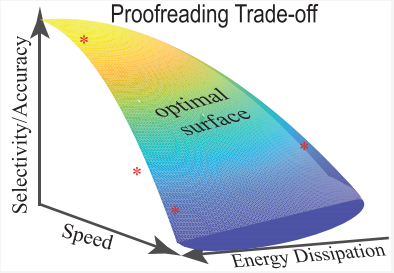
ABSTRACT: Most cellular processes involved in biological information processing display a surprisingly low error rate despite the stochasticity of the underlying biochemical reactions and the presence of competing chemical species. Such high fidelity is the result of nonequilibrium kinetic proofreading mechanisms, i.e., the existence of dissipative pathways for correcting the reactions that went in the wrong direction. While proofreading was often studied from the perspective of error minimization, a number of recent studies have demonstrated that the underlying mechanisms need to consider the interplay of other characteristic properties such as speed, energy dissipation, and noise reduction. Here, we present current views and new insights on the mechanisms of error-correction phenomena and various trade-off scenarios in the optimization of the functionality of biological systems. Existing challenges and future directions are also discussed.



INTRODUCTION

A remarkable property of enzymes operating inside the cell is their high selectivity, i.e., their ability to successfully discriminate between right and wrong substrates. This is especially true for the enzymes involved in the propagation of genetic information through the processes of the central dogma of biology: DNA to RNA to proteins.[1−3]

For example, it is known that DNA replication, which is a process of producing two identical DNA chains from a single DNA chain during cell divisions, has a very low error rate of $\eta \simeq 10^{-8}-10^{-10}$.[4] This result suggests that, amazingly, in the process of synthesizing a new DNA chain consisting of 10^8-10^{10} monomers, only a single mismatched (wrong) nucleotide is inserted (on average), while the concentrations of different nucleotides in the cellular medium are comparable.[1,2] Other biological processes also exhibit high selectivity, although it is not as impressive as that for DNA replication. Experimental observations show that RNA transcription, the first step of the transfer of genetic information when DNA is copied into a corresponding RNA molecule, has an error rate of $\eta \simeq 10^{-4}-10^{-5}$, while protein translation, the second step of the transfer of genetic information, has an error rate on the order of $\eta \simeq 10^{-3}-10^{-4}$.[5,6] Another example comes from T cells, which play a central role in the immune response of living organisms. These cells can accurately distinguish between the foreign and self-peptides with an error rate as small as $\eta \simeq 10^{-5}-10^{-6}$ despite a tremendous excess of self-to-nonself peptides.[7,8] There are many other biological processes, including protein ubiquitination and degradation,[9,10] signal transduction,[11] gene regulation,[12] and sensory adaptation[13] that exhibit highly specific outcomes, suggesting that error-correction processes are also involved in these systems.

Classical Picture of Enzyme Selectivity. The high selectivity of biological processes is surprising because they are taking place in a complex dynamically changing environment. There are multiple chemical species with similar properties that can participate in the same biochemical reactions, and it is expected that this should decrease the selectivity. In DNA replication, the correctly inserted nucleotide on one strand will make the corresponding hydrogen bonds with the base on the other strand. If the wrong nucleotide is inserted, it is expected that the interactions between the mismatched bases would be weaker by the order of the hydrogen bond strength $E_{HB} \sim 5-7k_BT$. This is because different nucleotides have chemical similarities and the hydrogen bonds are the main connecting elements for bonds between nucleotides on different DNA strands.[14−17] If the selection of right versus wrong nucleotides would be an equilibrium process, one could easily evaluate the error rate using Boltzmann's factors, $\eta_{equil} \simeq \exp[-E_{HB}/k_BT]$, producing $\eta_{equil} \simeq 10^{-2}-10^{-3}$. The fact that the observed selectivity is several orders of magnitude higher clearly shows that the differences in equilibrium binding stability of the correct and the mismatched substrates cannot explain very low error rates in the biological processes. Thus, the high fidelity is achieved due to the nonequilibrium nature of biological systems.

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The explanation of how the biological systems can achieve and sustain such surprisingly low error rates was proposed independently by Hopfield and Ninio more than 40 years ago.[18,19] It was suggested that there are additional biochemical pathways that can reset the system to its original state before reaching the final product, effectively lowering the probability of making the mistakes in the biological processes. To keep the net resetting flux from vanishing due to relaxation to equilibrium, the loops in the biochemical networks containing these transitions must dissipate energy, e.g., by hydrolysis of nucleotides. This error-correcting procedure is known as a kinetic proofreading mechanism (KPR).

To explain the main idea of the KPR mechanism as originally suggested by Hopfield and Ninio,[18,19] we illustrate it schematically in Figure 1. One can start with the simplest

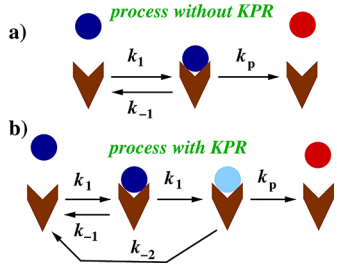

Figure 1. Schematic illustration of error-correction in enzymatic processes. Blue circles are substrate molecules, while red circles are product molecules. (A) Process without KPR that follows a simple Michaelis–Menten mechanism. (B) Process with KPR that has an additional intermediate state from which the system can reset back to the original state.

enzymatic process that follows the Michaelis–Menten mechanism with one intermediate enzyme–substrate complex (see Figure 1A). Both right and wrong substrates might follow this scheme, and if the substrate interactions with enzyme molecules are not very different, the high selectivity will be difficult to achieve. It was proposed[18,19] that inserting in the biochemical pathway one additional intermediate state from which the system can return back to the original state via a different pathway (Figure 1B) can significantly modify the error rate in the system. Due to this resetting, the enzymatic process with KPR, as shown in Figure 1B, will have more chances to distinguish between the right and wrong substrates, making the overall process highly selective. Critically, the resetting cycles must dissipate energy to keep the system away from equilibrium where no net fluxes are possible and the proofreading flux vanishes. These arguments suggest that KPR is a nonequilibrium process that utilizes additional biochemical pathways to achieve its goals.

The quantitative details of error-correction mechanisms in biological systems have been extensively debated from various points of view.[20−31] Many aspects of these phenomena, however, still remain not well-understood. In recent years, a clearer picture of the error corrections and of the overall optimization of biological systems started to emerge. From the original considerations that concentrated mostly on mechanisms of achieving high accuracy, the discussions shifted to evaluating the importance of other properties of the systems such as reaction speeds, energy dissipation, and noise reduction. Our goal is to briefly present the current views and new insights on the error-correction mechanisms and to discuss existing questions and challenges.

NEW INSIGHTS ON THE ERROR-CORRECTION MECHANISMS

Trade-Offs between Characteristic Properties of Biological Systems. KPR enhances the accuracy of biological processes by returning them to their original configurations without progressing to the final state (Figure 1B). However, these resetting events obviously slow down the speed of product formation, and this might be detrimental. Cells are complex dynamic systems with various processes that follow sequentially one after another one. If one process is not properly accomplished, the next one is delayed. This delay could have lethal consequences for the survival of the organism. These arguments stimulated the idea that there must be a trade-off between the speed and accuracy in biological processes.[11,20,23] In these studies, it was shown theoretically that the maximal accuracy might be achieved only when the reaction rate for the process goes to zero. For this reason, it was suggested that the biological systems most probably evolved to function under conditions when there is always a trade-off between speed and accuracy.[20,29,32] On the basis of available experimental data, the specific arguments were mostly presented for the ribosomes that are responsible for protein synthesis in cells.[23] However, some of the analysis employed an oversimplified Michaelis–Menten scheme and does not properly account for the complexity of biochemical mechanisms.

To understand better the trade-offs in error-correction processes, a general discrete-state stochastic framework was recently developed.[22,33] It is based on explicit calculations of dynamic properties using a method of first-passage probabilities.[34−36] Biological systems with a single resetting transition (so-called one-loop models), as illustrated in Figure 2, have been analyzed for general sets of transition rates for the

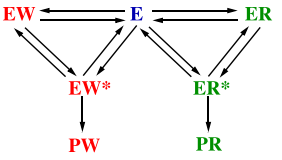

Figure 2. Schematic view of the one-loop biochemical network of transitions with KPR. There is a free-enzyme state E, from which the system can go along the right pathway to make the first enzyme substrate state ER and the second substrate-enzyme state ER* before reaching the right product state PR. From the free-enzyme state E the system can also move into the wrong pathway via the corresponding intermediate states EW and EW* to reach the wrong product PW. From each intermediate state, the system can always reset back to the free-enzyme state without reaching the product.

right (R) and wrong (W) pathways that were studied originally,[22,31,33,37] but the approach was also extended later to more complex biochemical schemes.[38]

The central part of this method is the analysis of the first-passage probability densities $F_{R,E}(t)$ and $F_{W,E}(t)$, which are defined as the probability to reach, for the first time, the product states PR and PW, respectively, at time t if the system started at $t = 0$ in the free-enzyme state E (see Figure 2) before the other product molecule is created.[22] These first-passage probability functions are calculated using so-called backward master equations[34−36] that describe their temporal evolution. All dynamic properties of the system can be expressed as moments of these functions. For example, the error rate η is

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defined as the ratio of the probabilities to reach the states PW and PR, which are also known as splitting probabilities[22]

$$\eta = \frac{\Pi_W}{\Pi_R} \qquad \Pi_{R/W} \equiv \int_0^\infty F_{R/W}(t)\, dt \tag{1}$$

The physical meaning of this expressions is clear: The error rate is larger if the probability to go along the wrong pathway is larger. Another advantage of the first-passage method is that the evaluation of the accuracy of the biological process is simultaneously accompanied by the calculation of the overall reaction rates, which are determined as the inverse mean first-passage times (MFPT) to reach the specific products. These times can be calculated from the first-passage probabilities[22]

$$\tau_{R/W} \equiv \langle t \rangle = \frac{\int_0^\infty t F_{R/W}(t)\, dt}{\Pi_{R/W}} \tag{2}$$

This theoretical framework was applied for two specific biological systems, namely, DNA replication by T7 DNA polymerases[4] and protein synthesis by *E. coli* ribosomes.[23,39] In both of these examples, the individual kinetic transition rates can be estimated from experimental measurements. To understand the trade-offs in DNA replication and protein translation, estimated kinetic parameters were used to compute the accuracy and reaction times, and then, their dependence on each individual reaction rate was investigated. Unexpectedly, it was found that in both cases the experimentally measured parameters correspond to nearly maximal reaction speed but not to the lowest error rate. In other words, evolution tuned the kinetic properties of these enzymes toward optimizing speed. Another surprising observation was that the speed−accuracy trade-off was not always observed as was previously assumed.

Therefore, one of the conclusions of the discrete-state stochastic analysis of ribosome and DNA polymerase trade-offs is that both enzymes employ a common strategy for the optimization.[22] It relies on enhancing the reaction speeds as long as the error rates are tolerable. It was suggested that the biological systems evolved to optimize their reaction times at the cost of accuracy. However, the tolerable level of accuracy is still needed, which is determined by the nature of the specific biological process, and this requirement constrains further optimization of the reaction speeds.[22]

The discussion of the optimization of biological processes raises a question regarding what the tolerable level of the error rate is. It can be argued that it is determined by the nature of the specific biological process as well as the genome size of the living organisms. The accuracy must be higher for the processes where mistakes could lead to more lethal consequences. This is the reason why experimentally observed DNA replication processes exhibit very low error rates ($\eta \simeq 10^{-8}-10^{-10}$).[4] However, the accuracy of RNA transcription and protein translation might be lower ($\eta \simeq 10^{-3}-10^{-5}$)[5,6] since mistakes during these processes are less dangerous for the survival of the organisms and can be corrected via alternative biochemical mechanisms. Recent studies also pointed out that the tolerable level of the error rate might correlate with the size of the genome, although the molecular underpinnings of these correlations still remain unclear.[40]

Although the speed and accuracy are important features of the enzymatic processes, it has been recognized for a long time that there are other characteristic properties that biological systems might optimize.[29,41−43] Notably, as indicated above, the proofreading requires the existence of energy-dissipating cycles, e.g., futile cycles of hydrolysis of energy-rich nucleotide triphosphate (NTP) molecules. This suggests that the correction of the errors in enzymatic processes might require significant energy consumption.[44−48] Because cellular resources are limited, this raised a question regarding if biological systems are also trying to minimize the energy dissipation.

To understand the role of energy cost in the error correction of biological systems, several theoretical studies investigated this issue from different points of view.[24,26,49,50] Using the second law of thermodynamics, a universal expression for the trade-off between the accuracy and energy dissipation (in terms of entropy production) has been derived.[49] It was shown that three different thermodynamic regimes of KPR are possible depending on how the energy is used for error amplification or error correction. However, only the entropy production from incorporation of the wrong substrates has been considered in this analysis. A complementary approach utilized a graph-based interpretation of biochemical networks to obtain universal scaling relations between energy dissipation, reaction speed, and accuracy.[24] With the application of the analysis to DNA replication by T7 DNA polymerases[4,22] and protein synthesis by *E. coli* ribosomes,[22,23,39] it was suggested that evolution apparently optimized more strongly the energy dissipation, speed, and accuracy for the replication processes; i.e., these properties are closer to their expected minimal or maximal values in comparison to the translational processes.[24] However, the presented calculations are based on asymptotic analysis, and this might explain why all of the experimental data did not follow the suggested scaling bounds for energy dissipation, speed, and accuracy.

The importance of dissipation in error-correction processes was also observed in the theoretical study of the single-loop model of KPR with a realistic constraint of fixed chemical potential over the resetting cycle.[31] Using a discrete-state stochastic description with first-passage calculations, it was found that for tRNA selection during protein synthesis the process is taking place at almost the maximum possible catalytic rate and entropy production rate, while the accuracy is far away from the maximum possible values. In addition, the system proceeds forward with the minimal energy consumption in the proofreading cycle. The observation of the overall maximal entropy production in this study contrasts with other theoretical works that argue for the minimal energy dissipation.[26,37] Thus, more studies are needed to understand the source of this difference.

A different method to investigate the trade-off between the energy dissipation, accuracy, and reaction speeds has been proposed recently.[26,50,51] It was suggested that it is convenient to analyze how the biological processes balance their properties by utilizing a recently obtained fundamental result, which is known as a thermodynamic uncertainty relation (TUR).[52] TUR quantifies the trade-off between energetic cost and precision of a nonequilibrium dynamic process in the stationary state, and the following bound is given[26,51,52]

$$Q = q(t)\epsilon_X^2(t) \geq 2k_B T \tag{3}$$

In this inequality, $q(t)$ presents the energy dissipation of generating a dynamic trajectory with output observable $X(t)$, and $\epsilon_X^2(t) \equiv \langle \delta X(t)^2 \rangle / \langle X(t) \rangle^2$ is the normalized variance in the output variable $X(t)$, which can be related to the error in the process. The idea here is that the larger variance corresponds

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to the larger error. For biochemical processes with KPR, the amount of the right product produced before time t might be used as a dynamic observable; i.e., $X(t) \equiv J_R t$.[26] The physical meaning of TUR is that to make the output of the dynamic process more precise it takes more energy, and eq 3 gives the physical limit of the precision for a given energy dissipation. A related recent study[51] argued that the most ideal situation for energy dissipation is found when there are only states and paths leading to the formation of the correct product. Then, it was found that for real systems T7 DNA polymerases operate close to this ideal limit, while ribosomes function ∼5 times farther from its ideal bound. This difference was assigned to the enhanced binding discrimination of the polymerases.

With an investigation of error-correction strategies using TUR analysis, it was shown that DNA replication by T7 DNA polymerases and RNA translation by *E. coli* ribosomes reduce their error rates and optimize to some degree their energy dissipation.[26] It was found that $Q \approx 10k_BT$ for T7 DNA polymerases and $Q \approx 45k_BT$ for *E. coli* ribosomes. While the observed dissipation levels are quite far from the theoretical limit of minimal dissipation ($2k_BT$), it was argued that they are suboptimized for given biological conditions. Although this study also finds that the reaction speeds are the most optimized features in these biological processes,[26] this approach concentrates mostly on the energy dissipation.

The discussions on possible optimization of various properties of biological systems such as speed, accuracy, and energy cost raised two additional questions.[37] First, although reaction rates, accuracy, and free-energy consumption are important characteristics for any biological system, it is not apparent whether there are other important characteristics of the enzymatic processes that are optimized by evolution. Second, it is not clear how the relative importance of each of these properties can be quantified and compared on a system level. To answer these questions, the original single-loop discrete-state stochastic models with first-passage analysis have been investigated to analyze together the error rate, reaction speed, energy dissipation, and noise.[37] The noise was interpreted as a normalized deviation of the dynamic properties of the system from the average values due to the stochastic nature of the chemical reactions that are the foundation of all biological processes. For example, the noise in the reaction times is given by the coefficient of variation for the first-passage time[37]

$$\delta_\tau = \frac{\sqrt{\langle t^2\rangle - \langle t\rangle^2}}{\langle t\rangle} \tag{4}$$

where $\langle t\rangle$ is the MFPT and the second moment of the first-passage probability density can be calculated from

$$\langle t^2\rangle = \frac{\int_0^\infty t^2 F_{R/W}(t)\, dt}{\Pi_{R/W}} \tag{5}$$

To quantify the degrees of optimization, a specific metric $d_{k,i}$ that measures the relative distance from the specific value of the property at a given set of natural chemical transition rates to the optimal possible value, if one of the rates k_i is varied, has been introduced. Then, the average number of the metric across all of the kinetic rates was employed, $D_{mean} \equiv \frac{1}{N}\sum_{i=1}^{N} d_{k,i}$. With the application of this theoretical approach for DNA replication by T7 DNA polymerases and for protein translation by *E. coli* ribosomes, overall rankings for different degrees of optimization have been obtained. The results of this comparison are presented in Figure 3. In both

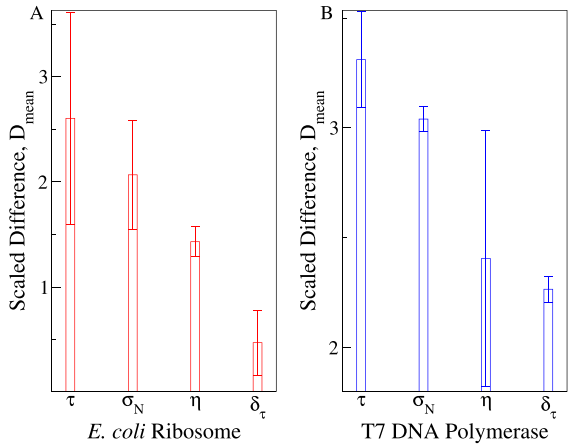

Figure 3. Relative rankings D_{mean} (see text for definition) of the degrees of optimization for reaction speed, energy dissipation, accuracy, and noise: (A) for translation by *E. coli* ribosomes and (B) for DNA replication by T7 DNA polymerases. Reprinted with permission from ref 37. Copyright 2019 American Chemical Society.

biological systems, it was found that the optimization of the speed is the most important criterion, closely followed by the optimization of the energy dissipation. At the same time, the error and the noise were always ranked third and fourth, respectively, underscoring the idea that these features are less crucial for the efficient functioning of the biological systems.

The above-described studies revealed that biological processes with a single proofreading step cannot simultaneously optimize reaction speed, accuracy, and energy consumption, and probably this was the reason that evolution prioritized specific characteristics.[22,24] It is, however, unclear how the optimization is taking place in more complex biological systems with multiple proofreading steps[5,53,54] when there are several resetting transitions in the underlying biochemical pathways.[29,55] This problem has been recently quantitatively investigated for isoleucyl-tRNA synthetase (IleRS) enzymes in *E. coli* bacteria,[38] which is one of the best characterized biological systems with multiple proofreading transitions.[56] The role of IleRS is to connect the correct tRNA molecule with the isoleucine amino acid during protein synthesis.[56] There are at least two different proofreading steps in the biochemical network of states for this enzymatic process. To analyze this system, a discrete-state stochastic framework with first-passage probability calculations[22] has been extended and generalized.[38] Surprisingly, it was found that because of the trade-off between the speed and energy dissipation the system adopted a so-called "economic" strategy in correcting errors. In one step, the reaction speed was optimized while in the other step the energy dissipation was minimized. This strategy allowed the system to avoid the conflicting optimization demands between the speed and energy consumption. The results are illustrated in Figure 4 where one can see that the system is optimizing reaction times and energy dissipation at the cost of accuracy. Interestingly, biochemical transformations in IleRS are taking place in different protein domains, and the existence of multiple proofreading pathways improves the overall functioning of this enzyme by relaxing the trade-off conflicts. One might suggest

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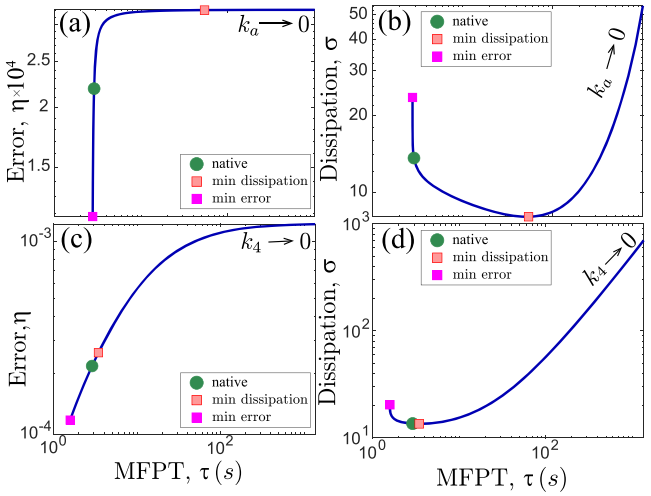

Figure 4. Observations of trade-offs between reaction times (MFPT, τ), error rates (η), and energy dissipation (σ) for isoleucyl-tRNA synthase enzymes in *E. coli* bacteria. The results are obtained by varying the rate constants of two catalytic steps: amino acid activation k_a coupled to ATP hydrolysis (upper panels) and amino acid transfer k_4 (bottom panels). The green dot corresponds to the native system, while the pink and magenta dots describe the positions of the minimum energy dissipation and error, respectively. Reprinted with permission from ref 38. Copyright 2020 American Chemical Society.

that one of the reasons why evolution favored multidomain proteins is to optimize the error-correction processes.

With all these observations in combination, the following microscopic picture of error-correction mechanisms in biological systems is emerging. In order to reach the most productive behavior, the systems prefer optimization of the reaction speeds and to a lesser degree the free-energy dissipation as long as the error rates are tolerable. In situations when there is a strong conflict between minimization of the reaction times and energy dissipation, the biological systems employ multiple steps and are able to optimize separately these important characteristics. In addition, it was found that trade-offs between different properties might not always exist.

New Perspective: Kinetic versus Thermodynamic Control of Error-Correction Processes. Starting from the original work of Hopfield,[18] it has been assumed that the enzyme selectivity is based on the higher affinity of the cognate substrate to the enzyme and the resultant differences in the dissociation rates (e.g., k_{-1} and k_{-2} in Figure 1 were assumed to be smaller for the right substrate while the remaining parameters were assumed to be the same). This interpretation corresponds to an energetic (thermodynamic) discrimination; i.e., the error is determined by the difference in the stabilities of the complexes for right or wrong substrates. On the other hand, the investigation of Bennett on copolymerization processes suggested that the error correction is governed only by kinetic factors.[41] These different views were later reconciled by a study,[28,57] which argued that both mechanisms are possible depending on the specific values of the transition rates. It was predicted that for very low catalytic rates (rates of making the product) thermodynamic discrimination dominates, while for fast catalytic rates the kinetic discrimination is the main source of error correction.

However, the claim that both thermodynamic and kinetic discrimination might govern the error-correction processes depending on the range of chemical transition rates has been recently challenged.[33] Employing a first-passage analysis for Michaelis–Menten and single-loop KPR schemes for a general set of transitions rates, it was shown explicitly that the error rate, with or without proofreading, is always governed by kinetic discrimination. In other words, the accuracy of enzymatic processes can be changed only by varying the transition-state energies (maxima) on the underlying free-energy profiles, while modifying only the energies of intermediate states (minima on the free-energy profiles) will not lead to any changes in the error rate. Thermodynamic discrimination appears only as a degenerate case when energies of the complexes and transition states are changed together, i.e., when both kinetic and thermodynamic discrimination factors are the same. The discrepancy between refs 28 and 33 partially originates from a failure of ref 28 to account for the effect of changes in the catalytic rates when the energy landscape is perturbed.

Notably, this new perspective calls into question the energetic arguments presented in the Classical Picture of Enzyme Selectivity section. Given that the selectivity is affected by the differences in the transition-state energies for the right and wrong products, we can envision the situation in which the differences in these energies would greatly exceed the energy of a hydrogen bond. In fact, the transition-state energies are effectively determined by the interactions within the active site of an enzyme and, therefore, can be greatly affected by small changes in the substrate geometry.

Kinetic control of the error for Michaelis–Menten and KPR schemes turned out to be a special case of a more general fundamental relation for nonequilibrium stationary fluxes and, therefore, should hold for any kinetic scheme.[58] To prove this result, Mallory et al.[58] considered general biochemical networks with quasi-first-order transitions and showed that the ratios of any stationary fluxes are invariant to energy perturbations of the discrete chemical states (minima on the underlying free-energy landscape) and are only influenced by changes in energy barriers (maxima on the underlying free-energy landscape). Thus, the properties of the system that depend on these ratios are controlled only by kinetic and not thermodynamic factors. The error rate for the enzymatic processes can be viewed as a ratio of the fluxes in the wrong and right directions, and for this reason, it also must be governed by the kinetic discrimination. In addition, other important properties of the proofreading networks, such as flux-normalized energy dissipation, are also proportional to the ratios of fluxes and, therefore, are kinetically controlled.

This theoretical result has an important consequence for developing synthetic biological systems in which the better selectivity is required. Changing the thermodynamic features like substrate binding energies will not improve the accuracy of such systems if the energy barriers are not affected.

Challenges and Future Directions. Significant progress has been made in recent years on clarifying the microscopic picture of error-correction mechanisms in living systems. We now understand much better the priorities in the evolutionary optimization of the functionality of enzymatic processes. At the same time, multiple questions on how biological systems achieve their efficiency remain unanswered. Let us briefly discuss several of them.

Due to recent advances in experimental methods, and especially in single-molecule techniques, the biochemical networks that characterized various biological processes can now be described with a much higher resolution. This allowed

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researchers to propose multiple theoretical ideas on the error-correction mechanisms. However, almost all experimental measurements are done for prokaryotic enzymes. As a result, different theoretical ideas have been tested only for relatively simple bacterial systems, but what happens in more complex eukaryotic cells remains totally unexplored. Although it is reasonable to assume that similar optimization principles govern these systems, one might imagine that additional criteria might also be utilized for error correction in eukaryotes. There is a strong need for comprehensive studies on these more complex biological systems.

Another important issue is to understand what determines the tolerable level of the error rate in different biological systems. Of course, one could argue that DNA replication should have the highest possible accuracy because the errors at this stage might be lethal to the organism, while protein synthesis might not be very accurate since the cellular machinery removes wrong polypeptide chains and misfolded proteins without significant consequences for the organism. However, the question is what determines the specific amplitudes of the error rates and how they are related with the size of the organism's genome and the nature of the given enzymatic process. There are observations on the correlations between the rate of mutations (which is a measure of errors) and the genome size; however, clear understanding of this issue is still lacking.[40]

In developing biotechnology or synthetic biology applications, it could be important to understand what features of underlying enzymatic mechanisms lead to the minimal error rates. There are theoretical arguments that the highest accuracy is achieved for local discrimination when only *few* chemical kinetic transition rates for corresponding steps differ from each other.[55] At the same time, enzymatic processes with kinetic proofreading in real systems exhibit a global discrimination, i.e., when *all* corresponding transitions rates are different. It is important to understand the physical mechanisms that might lead to the lowest possible errors and how this limit can be achieved.

The way in which biological systems are able to correct errors and optimize their functioning remains one of the most fascinating multidisciplinary topics. Significant efforts have been invested in uncovering the details of these phenomena. We now have a much clearer view on many aspects of error-correction and proofreading processes. The number of unresolved issues and challenges, however, remains high. However, the directions for future studies are now more clear. It is important to combine experimental and theoretical methods in order to comprehensively investigate the mechanisms of error-correction processes in biological systems.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Alberts, B.; Bray, D.; Hopkin, K.; Johnson, A. D.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Essential Cell Biology*; Garland Science, Taylor and Francis Group: New York, NY, 2014.
(2) Lodish, H.; Berk, A.; Kaiser, C. A.; Krieger, M.; Scott, M. P.; Bretscher, A.; Ploegh, H.; Matsudaira, P.; et al. *Mol. Cell. Biol.*; Macmillan, 2008.
(3) Phillips, R.; Kondev, J.; Theriot, J.; Garcia, H. *Physical Biology of the Cell*; Garland Science, 2012.
(4) Kunkel, T. A.; Bebenek, K. DNA replication fidelity. *Annu. Rev. Biochem.* **2000**, *69*, 497−529.
(5) Zaher, H. S.; Green, R. Fidelity at the Molecular Level: Lessons from Protein Synthesis. *Cell* **2009**, *136*, 746−762.
(6) Reynolds, N. M.; Lazazzera, B. A.; Ibba, M. Cellular Mechanisms that Control Mistranslation. *Nat. Rev. Microbiol.* **2010**, *8*, 849−856.
(7) Alon, U. *An Introduction to Systems Biology: Design Principles of Biological Circuits*; CRC Press, 2019.
(8) Cui, W.; Mehta, P. Identifying feasible operating regimes for early T-cell recognition: The speed, energy, accuracy trade-off in kinetic proofreading and adaptive sorting. *PLoS One* **2018**, *13*, e0202331.
(9) Lu, Y.; Wang, W.; Kirschner, M. W. Specificity of the anaphase-promoting complex: a single-molecule study. *Science* **2015**, *348*, 1248737.
(10) Bard, J. A.; Bashore, C.; Dong, K. C.; Martin, A. The 26S proteasome utilizes a kinetic gateway to prioritize substrate degradation. *Cell* **2019**, *177*, 286−298.
(11) Swain, P. S.; Siggia, E. D. The role of proofreading in signal transduction specificity. *Biophys. J.* **2002**, *82*, 2928−2933.
(12) Blossey, R.; Schiessel, H. Kinetic proofreading of gene activation by chromatin remodeling. *HFSP J.* **2008**, *2*, 167−170.
(13) Lan, G.; Sartori, P.; Neumann, S.; Sourjik, V.; Tu, Y. The energy−speed−accuracy trade-off in sensory adaptation. *Nat. Phys.* **2012**, *8*, 422−428.
(14) Petruska, J.; Goodman, M. F.; Boosalis, M. S.; Sowers, L. C.; Cheong, C.; Tinoco, I. Comparison between DNA melting thermodynamics and DNA polymerase fidelity. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 6252−6256.
(15) Kool, E. T. Hydrogen bonding, base stacking, and steric effects in DNA replication. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 1−22.
(16) Privalov, P. L.; Crane-Robinson, C. Forces maintaining the DNA double helix. *Eur. Biophys. J.* **2020**, *49*, 315.
(17) Privalov, P. L. Physical basis of the DNA double helix. *J. Biophys. Struct. Biol.* **2020**, *8*, 1−7.
(18) Hopfield, J. J. Kinetic Proofreading: A New Mechanism for Reducing Errors in Biosynthetic Processes requiring High Specificity. *Proc. Natl. Acad. Sci. U. S. A.* **1974**, *71*, 4135−4139.
(19) Ninio, J. Kinetic Amplification of Enzyme Discrimination. *Biochimie* **1975**, *57*, 587−595.
(20) Johansson, M.; Lovmar, M.; Ehrenberg, M. Rate and accuracy of bacterial protein synthesis revisited. *Curr. Opin. Microbiol.* **2008**, *11*, 141−147.
(21) Wohlgemuth, I.; Pohl, C.; Mittelstaet, J.; Konevega, A. L.; Rodnina, M. V. Evolutionary optimization of speed and accuracy of decoding on the ribosome. *Philos. Trans. R. Soc., B* **2011**, *366*, 2979−2986.
(22) Banerjee, K.; Kolomeisky, A. B.; Igoshin, O. A. Elucidating Interplay of Speed and Accuracy in Biological Error Correction. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 5183−5188.
(23) Johansson, M.; Zhang, J.; Ehrenberg, M. Genetic code translation displays a linear trade-off between efficiency and accuracy of tRNA selection. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 131−136.
(24) Wong, F.; Amir, A.; Gunawardena, J. Energy-Speed-Accuracy Relation in Complex Networks for Biological Discrimination. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **2018**, *98*, 012420.
(25) Chiuchiú, D.; Tu, Y.; Pigolotti, S. Error-speed correlations in biopolymer synthesis. *Phys. Rev. Lett.* **2019**, *123*, 038101.
(26) Song, Y.; Hyeon, C. Thermodynamic cost, speed, fluctuations, and error reduction of biological copy machines. *J. Phys. Chem. Lett.* **2020**, *11*, 3136−3143.
(27) Galstyan, V.; Phillips, R. Allostery and Kinetic Proofreading. *J. Phys. Chem. B* **2019**, *123*, 10990−11002.
(28) Sartori, P.; Pigolotti, S. Kinetic versus energetic discrimination in biological copying. *Phys. Rev. Lett.* **2013**, *110*, 188101.
(29) Murugan, A.; Huse, D. A.; Leibler, S. Speed, Dissipation, and Error in Kinetic Proofreading. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 12034−12039.
(30) Qian, H. Phosphorylation energy hypothesis: open chemical systems and their biological functions. *Annu. Rev. Phys. Chem.* **2007**, *58*, 113−142.
(31) Banerjee, K.; Das, B.; Gangopadhyay, G. The guiding role of dissipation in kinetic proofreading networks: Implications for protein synthesis. *J. Chem. Phys.* **2020**, *152*, 111102.
(32) Wohlgemuth, I.; Pohl, C.; Mittelstaet, J.; Konevega, A. L.; Rodnina, M. V. Evolutionary Optimization of Speed and Accuracy of Decoding on the Ribosome. *Philos. Trans. R. Soc., B* **2011**, *366*, 2979−2986.
(33) Banerjee, K.; Kolomeisky, A. B.; Igoshin, O. A. Accuracy of Substrate Selection by Enzymes is Controlled by Kinetic Discrimination. *J. Phys. Chem. Lett.* **2017**, *8*, 1552−1556.
(34) Van Kampen, N. G. *Stochastic Processes in Physics and Chemistry*; Elsevier, 1992; Vol. *1*.
(35) Redner, S. *A Guide to First-Passage Processes*; Cambridge University Press, 2001.
(36) Kolomeisky, A. B. *Motor Proteins and Molecular Motors*; CRC Press, Taylor and Francis Group: Boca Raton, FL, 2015.

https://dx.doi.org/10.1021/acs.jpcb.0c06180
J. Phys. Chem. B 2020, 124, 9289−9296

(37) Mallory, J. D.; Kolomeisky, A. B.; Igoshin, O. A. Trade-offs between error, speed, noise, and energy dissipation in biological processes with proofreading. *J. Phys. Chem. B* **2019**, *123*, 4718−4725.
(38) Yu, Q.; Mallory, J. D.; Kolomeisky, A. B.; Ling, J.; Igoshin, O. A. Trade-Offs between Speed, Accuracy, and Dissipation in tRNAIle Aminoacylation. *J. Phys. Chem. Lett.* **2020**, *11*, 4001−4007.
(39) Zaher, H. S.; Green, R. Hyperaccurate and Error-Prone Ribosomes exploit Distinct Mechanisms during tRNA Selection. *Mol. Cell* **2010**, *39*, 110−120.
(40) Lynch, M.; Ackerman, M. S.; Gout, J.-F.; Long, H.; Sung, W.; Thomas, W. K.; Foster, P. L. Genetic drift, selection and the evolution of the mutation rate. *Nat. Rev. Genet.* **2016**, *17*, 704.
(41) Bennett, C. H. Dissipation-Error Tradeoff in Proofreading. *BioSystems* **1979**, *11*, 85−91.
(42) Blomberg, C.; et al. Energy considerations for kinetic proofreading in biosynthesis. *J. Theor. Biol.* **1981**, *88*, 631−670.
(43) Savageau, M. A.; Lapointe, D. S. Optimization of kinetic proofreading: a general method for derivation of the constraint relations and an exploration of a specific case. *J. Theor. Biol.* **1981**, *93*, 157−177.
(44) Qian, H.; Kjelstrup, S.; Kolomeisky, A. B.; Bedeaux, D. Entropy Production in Mesoscopic Stochastic Thermodynamics: Nonequilibrium Kinetic Cycles Driven by Chemical Potentials, Temperatures, and Mechanical Forces. *J. Phys.: Condens. Matter* **2016**, *28*, 153004.
(45) Pigolotti, S.; Neri, I.; Roldán, É.; Jülicher, F. Generic Properties of Stochastic Entropy Production. *Phys. Rev. Lett.* **2017**, *119*, 140604.
(46) Gingrich, T. R.; Horowitz, J. M.; Perunov, N.; England, J. L. Dissipation Bounds All Steady-State Current Fluctuations. *Phys. Rev. Lett.* **2016**, *116*, 120601.
(47) Hyeon, C.; Hwang, W. Physical Insight into the Thermodynamic Uncertainty Relation using Brownian Motion in Tilted Periodic Potentials. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **2017**, *96*, 012156.
(48) Bel, G.; Munsky, B.; Nemenman, I. The Simplicity of Completion Time Distributions for Common Complex Biochemical Processes. *Phys. Biol.* **2010**, *7*, 016003.
(49) Sartori, P.; Pigolotti, S. Thermodynamics of error correction. *Phys. Rev. X* **2015**, *5*, 041039.
(50) Hwang, W.; Hyeon, C. Energetic costs, precision, and transport efficiency of molecular motors. *J. Phys. Chem. Lett.* **2018**, *9*, 513−520.
(51) Piñeros, W. D.; Tlusty, T. Kinetic Proofreading and the limits of thermodynamic uncertainty. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **2020**, *101*, 022415.
(52) Barato, A. C.; Seifert, U. Thermodynamic Uncertainty Relation for Biomolecular Processes. *Phys. Rev. Lett.* **2015**, *114*, 158101.
(53) Cvetesic, N.; Bilus, M.; Gruic-Sovulj, I. The tRNA A76 hydroxyl groups control partitioning of the tRNA-dependent pre-and post-transfer editing pathways in class I tRNA synthetase. *J. Biol. Chem.* **2015**, *290*, 13981−13991.
(54) Dulic, M.; Perona, J. J.; Gruic-Sovulj, I. Determinants for tRNA-dependent pretransfer editing in the synthetic site of isoleucyl-tRNA synthetase. *Biochemistry* **2014**, *53*, 6189−6198.
(55) Murugan, A.; Huse, D. A.; Leibler, S. Discriminatory Proofreading Regimes in Nonequilibrium Systems. *Phys. Rev. X* **2014**, *4*, 021016.
(56) Ling, J.; Reynolds, N.; Ibba, M. Aminoacyl-tRNA synthesis and translational quality control. *Annu. Rev. Microbiol.* **2009**, *63*, 61−78.
(57) Pigolotti, S.; Sartori, P. Protocols for copying and proofreading in template-assisted polymerization. *J. Stat. Phys.* **2016**, *162*, 1167−1182.
(58) Mallory, J. D.; Kolomeisky, A. B.; Igoshin, O. A. Kinetic control of stationary flux ratios for a wide range of biochemical processes. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 8884−8889.

https://dx.doi.org/10.1021/acs.jpcb.0c06180
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