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Article

Insights into Error Control Mechanisms in Biological Processes: Copolymerization and Enzyme-Kinetics Revisited

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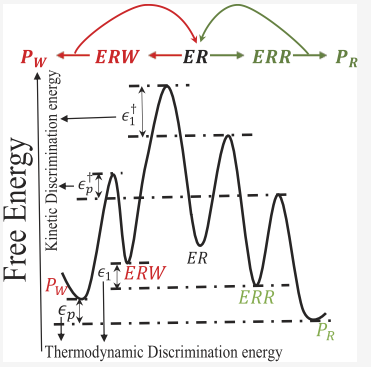
ABSTRACT: The high fidelity observed in biological information processing ranging from replication to translation has stimulated significant research efforts to clarify the underlying microscopic picture. Theoretically, several approaches to analyze the error rates have been proposed. The copolymerization theory describes the addition and removal of monomers at the growing tip of a copolymer, leading to a closed set of nonlinear equations. On the other hand, enzyme-kinetics approaches formulate linear equations of biochemical networks, describing transitions between discrete chemical states. However, it is still unclear whether the error values computed by the two approaches agree. Moreover, there are conflicting interpretations on whether the error is under thermodynamic or kinetic discrimination control. In this work, we examine the error rate in persistent copying biochemical processes by specifically analyzing both theoretical approaches. The initial disagreement of the results between the two theories motivated us to rederive the formula for the error rate in the kinetic model. The error computed with the new method resulted in excellent agreement between both theoretical approaches and with Monte Carlo simulations. Furthermore, our theoretical analysis shows that the kinetic discrimination controls the error, even when the energy difference between adding the right and wrong products is very small. Our theoretical investigation gives important insights into the physical−chemical properties of complex biological processes by providing the quantitative framework to evaluate them.



INTRODUCTION

Copolymerization is a process of chemically combining distinct monomer species to form polymer molecules.[1−4] Copolymerization processes can be classified into two categories: free copolymerization[5,6] and templated copolymerization that uses a template to control the distribution of produced sequences. Many biological information phenomena ranging from replication to translation rely on the templated copolymerization processes.[7−11] For instance, during DNA replication, different monomer nucleotides, either cognate or noncognate, are added sequentially to the growing DNA strand, following the sequence of the template DNA.[9,12] Therefore, the fidelity of the information processing can be understood by investigating the templated copolymerization kinetics.

One of the widely used approaches to study copolymerization kinetics is the Markov-chain copolymerization theory.[5,13−19] It assumes that a copolymer sequence can be described as a *k*th order Markov-chain with the rates of addition and removal of a monomer at the growing tip depending on the last *k* monomers before the growing tip.[20] For many biological processes, such as DNA replication, the addition and the removal reaction rates depend only on the last monomeric unit at the tip of the copolymer chain. For these processes, the copolymerization theory describes the copolymer sequences by a first-order Markov chain approach. In the literature, the copolymerization methods proposed by Krüger et al.,[13] Gaspard's method,[14] and the so-called steady-state copolymerization method,[15] utilize the first-order Markov chain for describing the copolymer sequences. This allows the reduction of hierarchically coupled kinetic equations that appear due to the complexities of reversibility or proofreading steps into a set of closed equations. These sets of nonlinear (in terms of probabilities of different states of the system) algebraic equations can be solved to obtain the stationary properties of the system, such as the error rate.

The enzyme-kinetics approach is another well-utilized method for analyzing the molecular mechanisms and the fidelity of biological information processing.[21−23] Enzymes are known to have remarkable capability for selectively choosing the right monomer and forming a right product, over a wrong monomer that forms a wrong product, during the binding of monomers to a substrate copolymer chain.[24] The enzyme-kinetics approach assumes that after the formation of either a right or a wrong product, the enzyme resets to its starting state, thus resulting in a discrete-state kinetic model.[25−27]

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This approach has been explored for the study of Hopfield kinetic proofreading (KPR) mechanisms and many other cellular processes that employ the KPR, such as DNA synthesis and repair, DNA replication, protein translation, receptor-initiated signaling, protein translation, protein folding, and transcription elongation.[9,24,27−35] In this method, the resultant steady-state kinetic equations, obtained either from backward or forward master equation formalism, are linear in terms of probabilities of individual chemical states.[23,24,31,34] In most cases, these equations can be solved analytically to obtain stationary probabilities, fluxes, and hence the error defined as the ratio of the stationary flux for forming a wrong product to the total stationary flux to form either a right or a wrong product.

The mechanisms of enzyme action can also be understood in terms of the free-energy landscape.[26,30,36−38] The differences in the free-energy profiles for the right and wrong products pathways chosen by an enzyme, determine the error rate. In general, the error rate and other nonequilibrium stationary properties can be governed by both kinetic discrimination, in which the differences in transition state energies (barriers to add right or wrong monomer) govern the process, and thermodynamic discrimination, in which the differences between the state energies for enzyme bound to right and wrong monomers determine the outcome of the process.

Recent studies of the kinetic models from the enzyme-kinetics approach proved that for a wide range of biochemical processes, such as protein folding, protein translation, motor protein transport, Michaelis−Menten enzymatic processes, and the Hopfield kinetic proofreading mechanism, the stationary flux ratios, and all properties that depend on them, such as error rate, are controlled by kinetic discrimination.[30,38] In other words, the error is invariant to the perturbations of the energies of the discrete states, and hence is only affected by the transition-state energies and their differences.[38] However, a recent study employing the Markov-chain copolymerization theory for the copolymerization processes suggested that the error is also subject to thermodynamic discrimination.[11]

The contradictory views on the kinetic and thermodynamic discrimination of the error obtained for two theoretical methods, the enzyme-kinetics, and the Markov-chain copolymerization, raise several questions, which we explore in this work. The first question is if the error rates computed from the two approaches are the same. If not, what is the microscopic origin of the discrepancy? If yes, what is the reason for the contrasting result for the control of kinetic versus thermodynamic discrimination on the error in biological processes? To answer these questions, we first present a theoretical analysis of information-copying processing using both copolymerization theory and enzyme-kinetics approaches. Then, the error rates from both methods are explicitly evaluated for the specific models for persistent copying processes such as DNA replication. For the kinetic model from the enzyme-kinetics approach, a new expression for error is obtained, showing that it now fully agrees with the results from the copolymerization theory. Using our quantitative frameworks, we analyze the conditions for thermodynamic and kinetic discrimination for determining their control on the error rate for the models for persistent copying processes. It is explicitly shown that the error in copolymerization processes is always governed by kinetic factors.

METHODS

Copolymerization Theory. To understand the kinetics of copolymerization, we use a first-order terminal model, in which the rates of addition and deletion of monomers in a copolymer sequence depend only on the previous monomer of the sequence.[14,15] Depending on whether the terminal monomer of any copolymer sequence is right (R) or wrong (W), there are two possible branching scenarios, as shown in Figure 1. In the figure, X represents a copolymer chain ending with monomer X. The addition Y (either R or W) to X creates the copolymer sequences ending with XY; the addition of Z (either R or W) to XY creates XYZ. The model reflects the templated copolymerization occurring in biological processes such as DNA replication and transcription. This scheme can also represent the free copolymerization process that does not rely on a template and is mainly used in industrial systems.[5,6,39] Based on the assumption that a copolymer sequence can be described as a first-order Markov chain, various theoretical methods, such as Krüger's approach and Gaspard's method, can be applied to reduce the original set of kinetic equations for the terminal model into the closed set of steady-state equations.[11,13,14] Below, we describe one such truncation method based on a Markov-chain approximation, named as steady-state copolymerization method, described in ref 15. It allows us to obtain the steady-state properties of the copolymerization process, such as stationary probability distributions, fluxes, and errors.

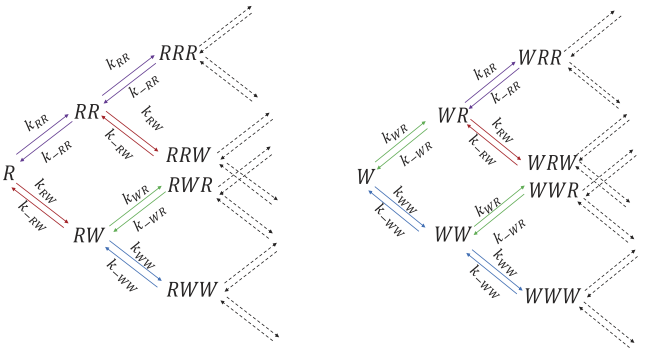

Figure 1. Two-step branching network of a growing copolymer chain with terminal unit R (left) and terminal unit W (right) in the first-order terminal model. X represents a copolymer chain ending with monomer X. XY represents the copolymer chain ending with dimer XY, and are formed by adding Y to the copolymer chain with X at the terminal position. Similarly, XYZ represents the copolymer chains ending with trimers XYZ, where, $X, Y, Z \in \{R, W\}$.

Let us define a chain-end sequence $i_n \cdots i_1$, where $i_k \in \{R, W\}$, $\forall k = 1, 2, \cdots n$, as representing a copolymer sequence with last n monomers at the growing end running from i_n to i_1, where i_1 being the terminal monomer, created by copying a specific template. If the monomer is copied correctly it has a value R in the sequence, while wrong copying is associated with W. We denote $P_{i_n \cdots i_1}(t)$ as the probability to have the copolymer sequence ending with $i_n \cdots i_1$ $(n \geq 1)$ at time t. The temporal evolution of these probabilities can be written using the flux conservation

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

$$\frac{dP_{i_n\cdots i_1}(t)}{dt} = J_{i_n\cdots i_1} - \tilde{J}_{i_n\cdots i_1 *} \tag{1}$$

where the fluxes are given as

$$J_{i_n\cdots i_1} = k_{i_2 i_1} P_{i_n\cdots i_2} - k_{-i_2 i_1} P_{i_n\cdots i_2 i_1} \tag{2}$$

$$\tilde{J}_{i_n\cdots i_1 *} = J_{i_n\cdots i_1 R} + J_{i_n\cdots i_1 W} \tag{3}$$

where we defined $k_{i_2 i_1}$ as the rate constant of the transition $i_n\cdots i_2 \rightarrow i_n\cdots i_2 i_1$ (adding the monomer to the chain) and $k_{-i_2 i_1}$ is the rate constant for the reversed transition $i_n\cdots i_2 i_1 \rightarrow i_n\cdots i_2$ (removal of the last monomer from the chain). Substituting eqs 2 and 3 into eq 1, we obtain

$$\begin{aligned}\frac{dP_{i_n\cdots i_1}}{dt} = &(k_{i_2 i_1} P_{i_n\cdots i_2} - k_{-i_2 i_1} P_{i_n\cdots i_2 i_1}) \\ &- (k_{i_1 R} P_{i_n\cdots i_2 i_1} - k_{-i_1 R} P_{i_n\cdots i_2 i_1 R}) \\ &- (k_{i_1 W} P_{i_n\cdots i_2 i_1} - k_{-i_1 W} P_{i_n\cdots i_2 i_1 W})\end{aligned} \tag{4}$$

The physical meaning of eq 4 is the following. The first term on the right side describes the transitions between the polymer configurations with $(n-1)$ and n last monomers, while the second term corresponds to $n \leftrightarrow (n+1)$ transitions if the last added monomer is the correct one (R) and the third term corresponds to $n \leftrightarrow (n+1)$ transitions if the last added monomer is the wrong one (W).

In the next important step, the probabilities of copolymer sequences ending with $i_n\cdots i_1$ $(n \geq 3)$ are approximated using the following factorization conjecture, based on the first-order Markov-chain approximation[14,15]

$$P_{i_n i_{n-1} i_{n-2}\cdots i_3 i_2 i_1} = \frac{P_{i_n i_{n-1}} P_{i_{n-1} i_{n-2}} \cdots P_{i_2 i_1}}{P_{i_{n-1}} P_{i_{n-2}} \cdots P_{i_2}} \tag{5}$$

This can also be understood as a two-site cluster mean-field approximation, meaning that the correlations inside the cluster of two neighboring monomers are considered explicitly, while the correlations with the sites beyond the cluster are neglected. Then, the probability of the arbitrary copolymer configuration can be viewed as a product of two-site probabilities (the numerator in eq 5) with the proper normalization coefficient (the denominator in eq 5).

Applying the conjecture from eq 5 into the expressions for the fluxes in eqs 2 and 3, we obtain

$$J_{i_n\cdots i_1} = \frac{\prod_{m=3}^{n} P_{i_m i_{m-1}}}{\prod_{m=4}^{n} P_{i_{m-1}}} \left(\frac{J_{i_2 i_1}}{P_{i_2}}\right) \tag{6}$$

and

$$\tilde{J}_{i_n\cdots i_1 *} = \frac{\prod_{m=2}^{n} P_{i_m i_{m-1}}}{\prod_{m=3}^{n} P_{i_{m-1}}} \left(\frac{\tilde{J}_{i_1 *}}{P_{i_1}}\right) \tag{7}$$

In the steady-state limit $(t \rightarrow \infty)$, we have $\frac{dP_{i_n\cdots i_1}}{dt} = 0$, leading to $J_{i_n\cdots i_1} = \tilde{J}_{i_n\cdots i_1 *}$. Then, from the eqs 6 and 7 one might conclude the following relation between the stationary fluxes and probabilities

$$\frac{J_{i_2 i_1}}{P_{i_2 i_1}} = \frac{\tilde{J}_{i_1 *}}{P_{i_1}} \tag{8}$$

Now using for the coefficients i_2 and $i_1 = R$ or W, it can be shown that

$$\frac{J_{RR}}{P_{RR}} = \frac{J_{WR}}{P_{WR}}, \qquad \frac{J_{RW}}{P_{RW}} = \frac{J_{WW}}{P_{WW}} \tag{9}$$

Additionally, for $i_1 = R$, the steady-state kinetic equation for P_{i_1}, yields $J_{RR} + J_{WR} = J_{RR} + J_{RW}$, finally leading to a simple relation.

$$J_{RW} = J_{WR} \tag{10}$$

The expressions for fluxes J_{RR}, J_{RW}, J_{WR}, and J_{WW}, can be obtained from eq 2, as follows.

$$J_{RR} = k_{RR} P_R - k_{-RR} P_{RR} \tag{11}$$

$$J_{RW} = k_{RW} P_R - k_{-RW} P_{RW} \tag{12}$$

$$J_{WR} = k_{WR} P_W - k_{-WR} P_{WR} \tag{13}$$

$$J_{WW} = k_{WW} P_W - k_{-WW} P_{WW} \tag{14}$$

Noting that the probability of a copolymer sequence ending with i_1 is equal to the sum of the probabilities of the copolymer sequences ending with Ri_1, and Wi_1, for $i_1 = \{R, W\}$, we obtain

$$P_R = P_{RR} + P_{WR}, \qquad P_W = P_{RW} + P_{WW} \tag{15}$$

Now substituting the expressions of fluxes from eqs 11−15 into the relations given by eqs 9 and 10, we obtain the following set of nonlinear algebraic equations

$$\begin{aligned}&(k_{-WR} + k_{RR} - k_{-RR}) P_{RR} P_{WR} + k_{RR} P_{WR}^2 - k_{WR} P_{RR} P_{WW} \\ &\quad - k_{WR} P_{RR} P_{RW} = 0 \\ &(k_{-WW} - k_{-RW} - k_{WW}) P_{RW} P_{WW} + k_{RW} P_{RR} P_{WW} \\ &\quad + k_{RW} P_{WR} P_{WW} - k_{WW} P_{RW}^2 = 0 \\ &k_{-WR} P_{WR} + k_{RW}(P_{RR} + P_{WR}) - k_{WR}(P_{RW} + P_{WW}) \\ &\quad - k_{-RW} P_{RW} = 0\end{aligned} \tag{16}$$

Solving the above-mentioned equations along with the normalization condition

$$P_{RR} + P_{RW} + P_{WR} + P_{WW} = 1 \tag{17}$$

yields the solutions of four unknown probabilities, P_{RR}, P_{RW}, P_{WR}, and P_{WW}. The specific values of these four variables can then be utilized for computing the error rate, η

$$\eta = \frac{J_{WR} + J_{WW}}{J_{RR} + J_{RW} + J_{WR} + J_{WW}} \tag{18}$$

It is defined as the ratio of stationary fluxes flowing into or out from the chains ending with the wrong monomer ($J_W = J_{WR} + J_{WW}$) to the sum of the stationary fluxes flowing into or out from the chains ending with monomer R ($J_R = J_{RR} + J_{RW}$) and monomer W (J_W).

Enzyme-Kinetics Approach. An alternative method to investigate the molecular mechanisms of information copying biological processes is the enzyme-kinetics approach.[21,22,25,28] Following this framework, we consider a minimal discrete-state kinetic model in which the forward and backward transition rates between any two states depend on both states. We, therefore, consider all possible states of the

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

enzyme (say) E that can occur before a product formation (one of the possible dimers: *rr*, *rw*, *wr*, *ww* at the copolymer tip) for a copolymerization process with rates depending on the previous monomer. A schematic view of relevant states and transitions in the enzyme-kinetics description is presented in Figure 2 with colored arrows indicating net fluxes between the states. If we assume that after the dimer product formation, the enzyme resets to the state corresponding the identity to the last incorporated monomer, we can describe the system with six-state kinetic model.

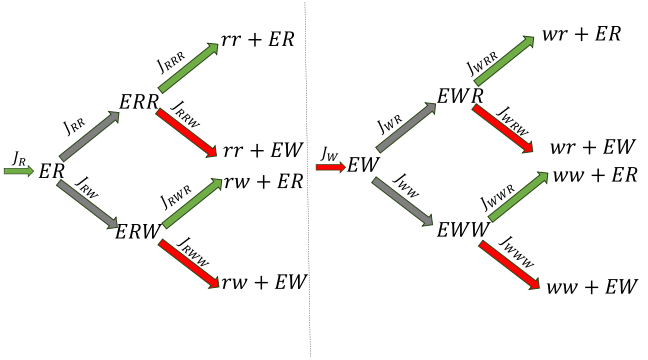

Figure 2. Relevant states and fluxes in the enzyme-kinetics description of the polymerization process. The system starts in either *ER* or *EW* states, where *E* represents the enzyme involved in the polymerization process, and then after two steps, ends up in one of these states along with the formation of the dimer products (*rr*, *rw*, *wr*, and *ww*).

Assuming that dynamics in the system are homogeneous and reach a stationary state, let us look at the copolymer sequence that ends at the site i at some arbitrary time: see Figure 2. If the last subunit is correctly copied, such state of the enzyme is labeled as *ER*, whereas if the last monomer is incorrectly copied, such state is labeled as *EW*. Then from *ER* and *EW*, four different enzyme states (*ERR*, *ERW*, *EWR*, *EWW*) can be achieved by adding another subunit (R or W) to the next site $i + 1$.

At the next step (site $i + 2$), in principle, there would be eight new possible states *EXYZ*, for $X, Y, Z \in \{R, W\}$. However, because the kinetic model assumes that the transition rate depends only on the chemical state of the last copied subunit, it is reasonable to restrict the network to six independent states. This is a periodic boundary condition that assumes that after the two steps enzyme resets to the state determined by the last incorporated monomer and forms the dimer products (*rr*, *rw*, *wr*, and *ww*); for details, see Figure 2.

The periodic boundary condition implies that the total flux flowing between the copolymer sequences ending with R (or W) at the sites $(i - 1)$ and i is equal to the total flux flowing between the copolymer sequences ending with R (or W) at sites $(i + 1)$ and $(i + 2)$ (see arrows with the same color in Figure 2). This leads to

$$J_R = J_{RRR} + J_{RWR} + J_{WRR} + J_{WWR} \tag{19}$$

$$J_W = J_{RRW} + J_{RWW} + J_{WRW} + J_{WWW} \tag{20}$$

where J_R and J_W are the total fluxes coming into the states, *ER* and *EW*, respectively, from the left. J_{XYZ} denotes the total flux flowing out of state *EXY* into the state *EXYZ*. Note that, $J_R = J_{RR} + J_{RW}$ (or $J_{RR} + J_{WR}$), and $J_W = J_{WR} + J_{WW}$ (or $J_{RW} + J_{WW}$), see (Figure 2), where

$$J_{RR} = k_{RR}P_{ER} - k_{-RR}P_{ERR} \tag{21}$$

$$J_{RW} = k_{RW}P_{ER} - k_{-RW}P_{ERW} \tag{22}$$

$$J_{WR} = k_{WR}P_{EW} - k_{-WR}P_{EWR} \tag{23}$$

$$J_{WW} = k_{WW}P_{EW} - k_{-WW}P_{EWW} \tag{24}$$

Notably, in contrast to the copolymerization theory, here, we view the six enzyme states, with probabilities P_i for $i \in \{ER, EW, ERR, ERW, EWR, EWW\}$, as independent states leading to the following normalization condition

$$P_{ER} + P_{EW} + P_{ERR} + P_{ERW} + P_{EWR} + P_{EWW} = 1 \tag{25}$$

The advantage of using the enzyme-kinetics approach to describe copolymerization is that the dynamics in the system can be viewed as a set of transitions between several discrete states with constant rates (as we assume that monomers and cofactors are at fixed concentrations). This allows us to utilize a powerful method of forward master equations (FME) to compute fluxes, probabilities, and other properties of the system

$$\mathbf{K}\cdot\mathbf{P} = \mathbf{0} \tag{26}$$

where the probability vector $\mathbf{P} = [P_{ER}, P_{ERR}, P_{ERW}, P_{EW}, P_{EWR}, P_{EWW}]$ is subject to the normalization condition (eq 25) rewritten in vector form

$$\mathbf{1}^{\mathrm{T}}\cdot\mathbf{P} = 1 \tag{27}$$

where $\mathbf{1}$ is a 6×1 unit vector. The 6×6 transition rates matrix $\mathbf{K}$, is given by

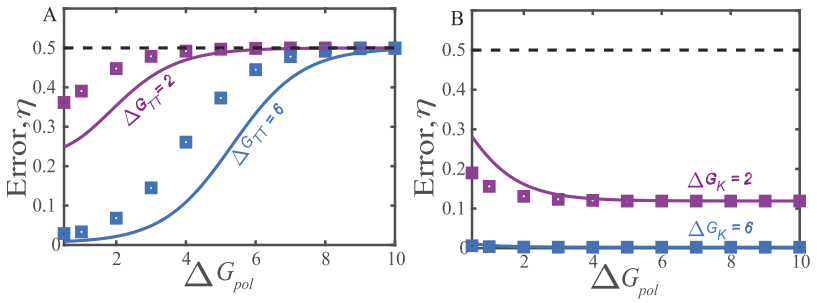

Figure 3. Error η as a function of ΔG_{pol} for (A) $\Delta G_K = 0$, and (B) $\Delta G_{TT} = 0$. Energies are given in units of k_BT. Solid lines correspond to the estimates from the enzyme-kinetics approach, and symbols correspond to the estimates from the copolymerization theory. The dashed lines represent the error for $\Delta G_K = \Delta G_{TT} = 0$ from both approaches.

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

$$\mathbf{K} = \begin{pmatrix} -D_1 & k_{-RR} + k_{RR} & k_{-RW} + k_{WR} & 0 & k_{RR} & k_{WR} \\ k_{RR} + k_{-RR} & -D_2 & 0 & k_{-RW} & 0 & 0 \\ k_{RW} + k_{-WR} & 0 & -D_3 & k_{-WW} & 0 & 0 \\ 0 & k_{RW} & k_{WW} & -D_4 & k_{-WR} + k_{RW} & k_{WW} + k_{-WW} \\ k_{-RR} & 0 & 0 & k_{WR} + k_{-RW} & -D_5 & 0 \\ k_{-WR} & 0 & 0 & k_{WW} + k_{-WW} & 0 & -D_6 \end{pmatrix} \tag{28}$$

where

$$D_1 = k_{RR} + 2k_{-RR} + k_{RW} + 2k_{-WR}$$
$$D_2 = k_{-RR} + k_{RR} + k_{RW}$$
$$D_3 = k_{-RW} + k_{WR} + k_{WW}$$
$$D_4 = 2k_{-RW} + 2k_{-WW} + k_{WR} + k_{WW}$$
$$D_5 = k_{RR} + k_{-WR} + k_{RW}$$
$$D_6 = k_{WR} + k_{WW} + k_{-WW} \tag{29}$$

Substituting the probabilities of the six distinct states into the expressions for stationary fluxes J_{RR}, J_{RW}, J_{WR}, and J_{WW}, given by eqs 21−24, and utilizing the definition of error as given by eq 18, the error rates for the enzyme-kinetics approach can be obtained.

Specific Models of Persistent Copying. To compare both theoretical approaches, let us consider a set of specific models of persistent copying (also see the Supporting Information) in which the transition rates can be related to the free-energy differences (in units of k_BT)[11]

$$k_{RR} = \exp(\Delta G_K), \qquad k_{-RR} = \exp(-\Delta G_{pol} + \Delta G_K) \tag{30}$$

$$k_{RW} = 1, \qquad k_{-RW} = \exp(-\Delta G_{pol} + \Delta G_{TT}) \tag{31}$$

$$k_{WR} = \exp(\Delta G_K), \qquad k_{-WR} = \exp(-\Delta G_{pol} + \Delta G_K - \Delta G_{TT}) \tag{32}$$

$$k_{WW} = 1, \qquad k_{-WW} = \exp(-\Delta G_{pol}) \tag{33}$$

where ΔG_{pol} defines the free-energy difference for extending a copolymer sequence by one monomer while ignoring the interactions between the copy and the template sequences (see Figure S5). The free energy, ΔG_{TT}, specifies the differences in interactions between the right monomer with the template and the wrong monomer with the template (see Figure S5). Here, we assume that only the last monomer interacts with the template while all previous contacts are already broken.[11] The kinetic energy, ΔG_K, denotes the difference between the transition state energies of adding the right or wrong monomers (see Figure S5). This implies that ΔG_K captures the kinetic advantage of adding the monomer R versus W.

Monte Carlo Simulations. To complement our theoretical approaches, we perform extensive Monte Carlo simulations (MCS) for the specific models of persistent copying defined above. We employed the Gillespie algorithm[40] to simulate the growth of a copolymer chain. The simulations are commenced with a random selection of a dimers sequence and are terminated upon reaching a copolymer length of 1000 monomers. The probability of errors is directly inferred from the outcomes of 1000 simulations. A length of 1000 monomers is chosen to avoid any edge effects in computing the probability of errors.

RESULTS

Discrepancy in Error Estimates for Enzyme-Kinetics and Copolymerization Methods. To compare the predictions for dynamic properties of information copying processes from both theoretical methods, let us start with evaluating the errors. The error rates are computed from the formula given by eq 18. The fluxes involved in the expression for the error are evaluated from eqs 11−14 for the copolymerization method and from eqs 21−24 for the enzyme-kinetics approach. At the same time, the probabilities in the expressions for fluxes are computed from the solution of eqs 9, 10, and 15 for the copolymerization theory; whereas for the enzyme-kinetics approach, the probabilities are obtained from solving the system of equations defined by eqs 8 and 13.

Figure 3 shows the results of our calculations, illustrating how the error rate changes as a function of polymerization energy, ΔG_{pol}, for the two cases. The first case assumes that $\Delta G_K = 0$ for different ΔG_{TT} (see Figure 3A), where all free energies are given in units of k_BT. This corresponds to the situation when there is no kinetic advantage of adding the monomer R versus monomer W. The second case is for $\Delta G_{TT} = 0$, i.e., when every final product state has the same free energy, for different ΔG_K (see Figure 3B). In both cases, there are discrepancies between the errors computed from the enzyme-kinetics approach (solid lines) and the copolymerization theory (symbols). However, for $\Delta G_{TT} = 0$, the discrepancies between the two theories are observed for a smaller range of parameters. Whereas, for the case when only $\Delta G_K = 0$, the errors from the two theories only match in the limit of the threshold value of 0.5, a condition achieved when both ΔG_K, and ΔG_{TT} are set to zero (dashed line in Figure 3).

To determine which of the two approaches is more accurate in estimating the error, we also compared the predictions with the error obtained from MCS (see Figure S4). It was found that the error from the copolymerization method always matches with the corresponding results from the simulations (see Figure S4). However, the error from the copolymerization theory matches with the error from the enzyme-kinetics approach only when the backward transition rates become small in comparison to the forward transition rates, that is, when ΔG_{pol} is large (see Figure 3). This is because the presence of backward transitions causes the probabilities of copolymer sequences ending with chains of

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

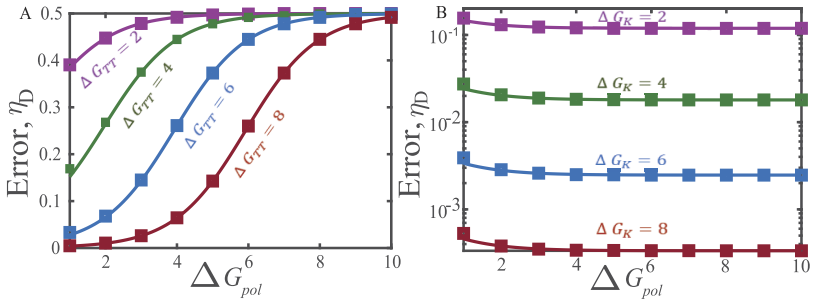

Figure 4. Error η_D as a function of ΔG_{pol} for two cases: (A) $\Delta G_K = 0$, and $\Delta G_{TT} = 2$, 4, 6, and 8. (B) $\Delta G_{TT} = 0$, and $\Delta G_K = 2$, 4, 6, and 8. Energies are given in units of k_BT. Solid lines denote the error for the enzyme-kinetics approach. Symbols denote the error obtained from the copolymerization theory.

length i to depend on the probabilities of copolymer sequences ending with chains of length $i + 1$ and $i - 1$, which is generally not fully accounted for in the enzyme-kinetics approach. These arguments suggest that the evaluation of the errors in the enzyme-kinetics method should be reconsidered to reflect properly for the backward transitions.

Modified Formula of Error for Enzyme-Kinetics Approach. To uncover the sources of the discrepancy in the estimates of errors from the enzyme-kinetics approach, we relate the fluxes to the conditional error probabilities, η_R or η_W, defined as the probability to have the monomer W at a position $i + 1$, given that a monomer R or respectively W is present at the ith position of the copolymer chain.[5] Equivalently, η_R (η_W) is the probability of arriving at the state ERW (EWW) at the position $i + 1$, given that the system is at the state ER (EW) at the ith position[5]

$$\eta_R = \frac{J_{RW}}{J_{RR} + J_{RW}} \tag{34}$$

$$\eta_W = \frac{J_{WW}}{J_{WR} + J_{WW}} \tag{35}$$

where J_{XY} denotes the flux between the states EX and EXY in the discrete-state network.

Let us define the total flux of adding the two monomers to the copolymer chain as $J_T = J_{RR} + J_{WR} + J_{RW} + J_{WW}$. One can present the fluxes J_{XY} in terms of parameters η, η_R, η_W, and J_T, as follows

$$J_{RR} = (1 - \eta)(1 - \eta_R)J_T \tag{36}$$

$$J_{RW} = (1 - \eta)\eta_R J_T \tag{37}$$

$$J_{WR} = \eta(1 - \eta_W)J_T \tag{38}$$

$$J_{WW} = \eta\eta_W J_T \tag{39}$$

In the same fashion, it can be shown that

$$J_{XYR} = J_{XY}(1 - \eta_Y) \tag{40}$$

$$J_{XYW} = J_{XY}\eta_Y \tag{41}$$

In addition, we note that $J_{XY} = J_{XYR} + J_{XYW}$, $\forall X, Y \in \{R, W\}$. This means that the net flux coming into the state EXY from the left is equal to the net flux going out of the state EXY into the right: see the discrete states network in Figure 2.

Substituting the fluxes J_{XY} and J_{XYZ} from eqs 36−41 into the expressions given by eqs 19 and 20 leads to simpler relations

$$(\eta_R - \eta(1 + \eta_R - \eta_W))(1 - \eta_R + \eta_W) = 0 \tag{42}$$

$$(\eta_R - \eta(1 + \eta_R - \eta_W))(\eta_R - \eta_W) = 0 \tag{43}$$

Solving either of the above equations for η, we derive the following relation of the error rate, denoted by η_D, for the discrete-state kinetic model

$$\eta_D = \frac{\eta_R}{1 + \eta_R - \eta_W} \tag{44}$$

This result can also be derived using much more intuitive physical arguments. One can view the error as a quantity average over two possible cases when the last monomer in the copolymer chain is R or W, namely

$$\eta = P_R\eta_R + P_W\eta_W \tag{45}$$

where P_R and P_W are the probabilities to have the last monomer R or W, respectively. One can associate $P_W = \eta$ and $P_R = 1 - \eta$, which after substitution into eq 45 leads to the expression in eq 44. These arguments suggest that the correct expression for the error should reflect different dynamics of adding or removal the R and W monomers depending on the nature of the last monomer in the copolymer chain. It should be noted that η_D is obtained in terms of the conditional error probabilities unlike the error η, given in eq 18.

Further substituting η_R, and η_W, from eqs 34 and 35 into eq 44, yields the final modified expression for the error in the enzyme-kinetics

$$\eta_D = \frac{J_{RW}(J_{WR} + J_{WW})}{J_{RR}J_{WR} + J_{RW}(2J_{WR} + J_{WW})} \tag{46}$$

One could notice that using the modified expression of error in eqs 37 and 38 leads to the condition

$$J_{RW} = J_{WR} \tag{47}$$

Equivalently, enforcing this condition implies that errors computed from eqs 46 and 18 are the same. For the steady-state copolymerization method, the condition given by eq 47 always holds (see the Methods section). However, for the enzyme-kinetics approach, the fluxes J_{RW} and J_{WR}, when computed from eqs 12 and 13, are not equal (see Figure S2 in the Supportingg Information). This implies that for the discrete-state kinetic model obtained from the enzyme-kinetic approach, the new formula for the error, η_D, is not always

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

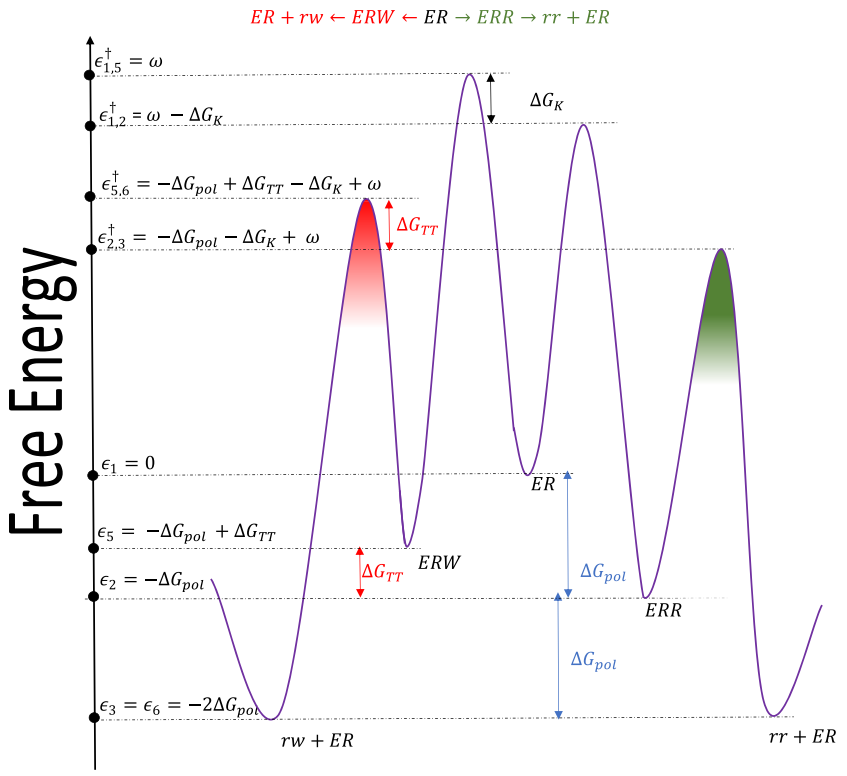

Figure 5. Effective free-energy landscape of copolymerization processes. Part of the overall network is shown.

equal to the original expression for the error given by eq 18. We also note that $J_{RW} = J_{WR}$ is the result of the conservation of total flux in the steady-state, which the enzyme-kinetic approach fails to capture.

To check the accuracy of the new error formula, we compute the error, η_D, for the persistent copying processes with the rates given by eqs 30−33, using the FME formalism for the enzyme-kinetics approach. For the case when $\Delta G_K = 0$, the error is given by

$$\eta_{D,K} = \frac{1 + e^{\Delta G_{TT}} + 4\, e^{(\Delta G_{pol} + \Delta G_{TT})}}{1 + 2\, e^{\Delta G_{TT}} + e^{2\Delta G_{TT}} + 8\, e^{(\Delta G_{pol} + \Delta G_{TT})}} \tag{48}$$

The above-mentioned error matches with the corresponding numerical values of error, computed from the steady-state copolymerization theory, for different values of ΔG_{TT} and a broader range of ΔG_{pol} as shown in Figure 4A. For the case of $\Delta G_{TT} = 0$, the error η_D is given by

$$\eta_{D,TT} = \frac{e^{\Delta G_K} + e^{\Delta G_{pol}} + e^{(\Delta G_K + \Delta G_{pol})}}{2\, e^{\Delta G_K} + e^{\Delta G_{pol}} + 2\, e^{(\Delta G_K + \Delta G_{pol})} + e^{2\Delta G_K + \Delta G_{pol}}} \tag{49}$$

It also agrees with the corresponding estimates of the error obtained from the copolymerization theory and from the computer simulations, as demonstrated in Figure 4B.

These observations suggest that the evaluation of the error for information copying processes in biological systems from both theoretical methods produces the same results that also fully agree with computer simulations. However, in the enzyme-kinetics method the calculation of the error is relatively simple due to the linear nature of underlying relations, leading to explicit analytical formulas. At the same time, calculations in the copolymerization approach are more complex because of the nonlinear nature of involved relations. As the result, it can only provide numerical estimates.

Invariance of Errors with Respect to Energy Perturbations. It is convenient to analyze the biological information copying processes in terms of the effective free-energy landscape where the minima correspond to specific biochemical states and the maxima describe the barriers for transitions between these states. Generally, free-energy perturbations should influence the dynamic properties of copolymerization.[41] However, it has been recently shown that the dynamic properties that depend only on the ratio of stationary fluxes should be independent of those perturbations that affect only the minima in the underlying free-energy profile while keeping all other energies unchanged.[38] This is known as a kinetic discrimination control because these properties can only be changed by modifying the kinetic rates for transitions between the states (i.e., changing the maxima in the free-energy landscape).

One can see that the modified expression for the error, η_D can also be rewritten as a function of the ratios of stationary fluxes

$$\eta_D = \frac{J_{WR} + J_{WW}}{J_{RR}\frac{J_{WR}}{J_{RW}} + 2J_{WR} + J_{WW}} = \frac{J_{WR}/J_{RR} + J_{WW}/J_{RR}}{J_{WR}/J_{RW} + 2J_{WR}/J_{RR} + J_{WW}/J_{RR}} \tag{50}$$

Since each ratio of stationary fluxes is invariant under the perturbations of state energies, the whole expression for η_D must also be invariant. This implies that the error cannot be controlled thermodynamically, and it depends only on the kinetic aspects of the process, that is, it is under kinetic discrimination control.

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

Table 1. Energies of Discrete States (Minima), ϵ_j, and Transition State Energies (Maxima), $\epsilon^{\dagger}_{i,j}$, of the Free-Energy Landscape of Copolymerization[a]

internal-state energies	value	transition-state energies	value
ϵ_2	$-\Delta G_{pol}$	$\epsilon^{\dagger}_{1,2}$	$\omega-\Delta G_K$
ϵ_3	$-2\Delta G_{pol}$	$\epsilon^{\dagger}_{1,5}$	ω
ϵ_4	$-2\Delta G_{pol} + \Delta G_{TT}$	$\epsilon^{\dagger}_{2,3}$	$-\Delta G_{pol} + \omega-\Delta G_K$
ϵ_5	$-\Delta G_{pol} + \Delta G_{TT}$	$\epsilon^{\dagger}_{2,4}$	$-\Delta G_{pol} + \omega$
ϵ_6	$-2\Delta G_{pol}$	$\epsilon^{\dagger}_{5,6}$	$-\Delta G_{pol} + \Delta G_{TT} + \omega-\Delta G_K$
ϵ_7, ϵ_{14}	$-2\Delta G_{pol} + \Delta G_{TT}$	$\epsilon^{\dagger}_{5,7}$	$-\Delta G_{pol} + \Delta G_{TT} + \omega$
ϵ_8	ΔG_{TT}	$\epsilon^{\dagger}_{8,9}$	$\Delta G_{TT} + \omega-\Delta G_K$
ϵ_9	$-\Delta G_{pol}$	$\epsilon^{\dagger}_{8,10}$	$\Delta G_{TT} + \omega$
ϵ_{10}	$-\Delta G_{pol} + \Delta G_{TT}$	$\epsilon^{\dagger}_{9,11}$	$-\Delta G_{pol} + \omega-\Delta G_K$
ϵ_{11}	$-2\Delta G_{pol}$	$\epsilon^{\dagger}_{9,12}$	$-\Delta G_{pol} + \omega$
ϵ_{12}	$-2\Delta G_{pol} + \Delta G_{TT}$	$\epsilon^{\dagger}_{10,13}$	$-\Delta G_{pol} + \Delta G_{TT} + \omega-\Delta G_K$
ϵ_{13}	$-2\Delta G_{pol}$	$\epsilon^{\dagger}_{10,14}$	$-\Delta G_{pol} + \Delta G_{TT} + \omega$

[a]The labels i describe different states in the network, as partially shown in Figure 5, and fully described in the Supporting Information and shown in Figure S3.

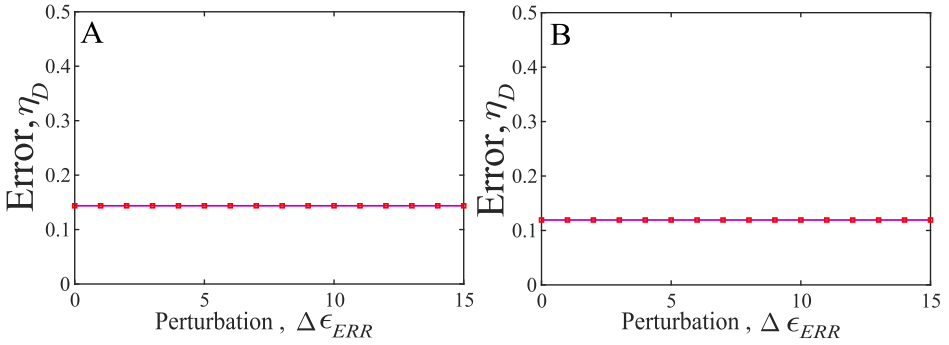

Figure 6. Error as a function of energy perturbation, $\Delta\epsilon_{ERR}$ for two cases (A) $\Delta G_K = 0$, $\Delta G_{TT} = 6$, $\Delta G_{pol} = 3$; (B) $\Delta G_K = 2$, $\Delta G_{TT} = 0$, $\Delta G_{pol} = 6$. Free energies are given in units of k_BT. Solid lines and symbols, respectively, denote the errors computed from the enzyme-kinetics approach and copolymerization theory.

Is Persistent Copying Error Controlled Thermodynamically or Kinetically? In dynamic processes with multiple different outcomes, the kinetic control describes the situation when the probabilities of getting different products depend only on the transition state energies and are independent of the state energies. Thermodynamic control corresponds to the opposite case when the probabilities of different products are governed only by the state energies and not by the transition barriers between the states. The copolymerization processes belong to such a class of dynamic systems with different products.

Recent theoretical investigation of persistent copying processes with the rates described by eqs 30−33 considered the situation when $\Delta G_K = 0$ as the case of no kinetic discrimination because the rates of adding right (R) or wrong (W) in this limit are the same.[11] Then, it was shown for this situation that errors depended on the variation of ΔG_{pol} and ΔG_{TT}, which can be viewed as thermodynamic quantities: see also Figure 3. Based on these observations, it was argued that errors in copolymerization processes are under thermodynamic control. However, this contradicts the theoretical arguments that we presented above that clearly show only the kinetic discrimination for errors in such biochemical processes.[38]

To resolve this controversy, let us use the free-energy landscape for the copolymerization process as illustrated in Figure 5. Assuming that the initial state ER has zero energy, we can assign specific values for the energies of discrete states (minima) and transition state energies (maxima) in terms of the energetic parameters of the models, ΔG_{pol}, ΔG_K, and ΔG_{TT}. The results are summarized in Table 1, and more details are given in the Supporting Information. Importantly, one can see that even when $\Delta G_K = 0$ most transition state energies also depend on ΔG_{pol} and ΔG_{TT}. This means that varying these energetic parameters changes both minima and maxima in the free energy profile, and this cannot be viewed as a pure thermodynamic control. Such perturbations simultaneously modify both the kinetic and thermodynamic features of the system.

To better understand why setting $\Delta G_K = 0$ does not correspond to thermodynamic discrimination conditions, one can visualize the free energy landscape for two subsequent incorporation steps starting from the same state ER and ending in either error-less product state $rr + ER$ or one wrong monomer incorporated product state $rw + ER$ (Figure 5). Both final states have the same energies equal to $-2\Delta G_{pol}$ (consistent with periodic boundary conditions imposed in eqs 19 and 20). One can see that while $\Delta G_K = 0$ results in the zero difference in the transition state energies (maxima) for the first reaction steps in both pathways: $R \rightarrow RR$ or $R \rightarrow RW$ for copolymerization (or $ER \rightarrow ERR$ or $ER \rightarrow ERW$ for the enzyme-kinetics approach), the transition state energies for the subsequent steps are not the same. Indeed, changing either ΔG_{pol} or ΔG_{TT} affects these transition state energies. Furthermore, if $\Delta G_{TT} \neq 0$, there will be a difference in these energies, and, therefore, the rates of following reaching rr or

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

rw products are expected to differ leading to the kinetic discrimination. Notably, if $\Delta G_K = \Delta G_{TT} = 0$ there will be no discrimination between the two substrates, and the error will be 0.5. Thus, we conclude that for the persistent copying processes with rates defined by eqs 30−33, the pure thermodynamic discrimination cannot be defined by merely putting $\Delta G_K = 0$.

To check the invariance of the errors computed from the enzyme-kinetics approach or copolymerization theory, the following procedure can be done. Consider an arbitrary discrete state of the system m with the energy, ϵ_m. Let us modify only this state energy by the amount $\Delta\epsilon_m$ such that $\epsilon'_m = \epsilon_m - \Delta\epsilon_m$. Then the transition state energies and state energies of all other states $i \neq m$ are fixed. This perturbation will modify the transition rates out of the state m, and the error can be again estimated explicitly in the perturbed system. Figure 6 demonstrates the error as a function of perturbation of energy of state *ERR* by an amount $\Delta\epsilon_{ERR}$ for the two cases: when $\Delta G_K = 0$ and when $\Delta G_K \neq 0$. It is found that, for both cases, the errors computed either for the enzyme-kinetics approach or from the copolymerization theory are invariant to such perturbations, implying that the error for the persistent copying processes is always under the kinetic discrimination control.

DISCUSSION

In our work, we compared two theoretical approaches, the copolymerization method and the enzyme-kinetics, to analyze the error rate of biological information processing. The copolymerization theory employs the factorization conjecture, which is equivalent to the Markov-chain approximation for the dependence of the addition and deletion rates of monomers at the growing tip on the last monomer of the chain. This approximation reduced the steady-state kinetic equations into a set of closed nonlinear algebraic equations. The solutions of these equations are the stationary probabilities, which can be used to compute the stationary fluxes and the error rate. On the other hand, the enzyme-kinetics approach assumes that the enzyme resets to one of the original states after the product is formed and, as a result, it yields a finite-state chemical-kinetic model. The resulting kinetic equations are linear, allowing us to obtain stationary probabilities and the fluxes that can be used to compute the error.

The error was evaluated using both theoretical approaches and compared with the MCS for the specific model of persistent copying. In this model, the rates of addition and deletion of monomers are defined in terms of driving energy ΔG_{pol}, kinetic difference energy ΔG_K, and temporary thermodynamic difference energy ΔG_{TT}.[11] The results indicate that the error computed from copolymerization theory agreed perfectly with MCS while some disagreements were found with the results computed from the enzyme-kinetics approach. This discrepancy in the error motivated us to rederive the error for the enzyme-kinetics approach. The modified error formula has been obtained by redefining the fluxes in terms of the conditional error probabilities. The new expression for the error agreed well with the error obtained from the copolymerization theory and with MCS.

We also investigated what factors govern the error rate in biological information copying processes. There are contradictory results suggesting only kinetic or only thermodynamic discrimination for error in persistent copying.[11,38] Our explicit analysis using free-energy calculations determined that the error rates are controlled only by the kinetic factors. It was also found that the perturbations that were used to claim for thermodynamic discrimination actually involved the modifications of both kinetic and thermodynamic features, and, thus, they yielded wrong predictions. Our calculations suggest that energetic perturbations that only change the state energies (minima in free-energy profile) do not influence the error rates, while changes in the transition state energies (maxima in free-energy profile) lead to changes in the errors.

Our calculations show that $\Delta G_K = 0$ (the same rate of adding right or wrong monomers) is not a regime of the pure thermodynamic discrimination. The explicit expressions for the energies and transition energies for the various states of the kinetic model indicated that some of the transition state energies explicitly depend on the polymerization energy ΔG_{pol} or thermodynamic discrimination energy ΔG_{TT}. Therefore, perturbations of these parameters will also change the kinetic properties of the system. For example, even when $\Delta G_K = 0$, the difference in the transition state energies for the reactions $ERR \rightarrow ERRR$ and $ERW \rightarrow ERWR$ is ΔG_{TT}. Thus, kinetic discrimination will only be absent when $\Delta G_{TT} = 0$ and, in fact, in this limit, both approaches result in the error of 0.5, that is, lack of discrimination.

In this work, we investigated the underlying molecular mechanisms of biological copying processes by analyzing them using two different theoretical methods, copolymerization theory, and enzyme-kinetics approach. This allows us to compare both theoretical methods, pointing out to strong and weak sides of each approach. The copolymerization theory provides a numerically exact estimate of the error. This is due to the use of the first-order Markov-chain approximation that properly captures the nearest-neighbor effect, that is, that the monomers association and dissociation rates depend only on the last subunit in the copolymer. In other words, the correlation length in this one-dimensional system is equal to the distance between the nearest neighbors. Moreover, for more complex copolymerization processes with internal substates, the theory can be generalized using the so-called Markov-chain tree theorem.[4] However, the copolymerization theory always produces a set of complex nonlinear kinetic equations that very rarely lead to analytical solutions, requiring advanced numerical calculations to obtain the dynamic properties. On the other hand, the enzyme-kinetic approach leads to simpler linear kinetic equations, from which analytical results can be obtained, significantly accelerating the efforts to understand the microscopic picture of persistent copying and related phenomena. In addition, although the simplest version of the enzyme-kinetic method assumes only few discrete states, more chemical states can be added in a more general approach. It seems that combining both theoretical methods can provide a powerful tool for investigations of complex biological processes.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.4c02173.

Detailed description of persistent copying process; free energy landscape description for the kinetic models for the persistent copying processes (PDF)

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J. Phys. Chem. B XXXX, XXX, XXX−XXX

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Mayo, F. R.; Walling, C. Copolymerization. *Chem. Rev.* **1950**, *46*, 191−287.

(2) Berlin, A. A.; Volfson, S. A.; Enikolopian, N. S. Kinetics of polymerization processes. *Polym. Processes* **1981**, *38*, 89−140.

(3) Boulding, N. A.; Millican, J. M.; Hutchings, L. R. Understanding copolymerisation kinetics for the design of functional copolymers via free radical polymerisation. *Polym. Chem.* **2019**, *10*, 5665−5675.

(4) Qureshi, B.; Juritz, J.; Poulton, J. M.; Beersing-Vasquez, A.; Ouldridge, T. E. A universal method for analyzing copolymer growth. *J. Chem. Phys.* **2023**, *158*, 104906.

(5) Gaspard, P. Kinetics and thermodynamics of living copolymerization processes. *Philos. Trans. R. Soc., A* **2016**, *374*, 20160147.

(6) Corfield, A. P. Mucins: a biologically relevant glycan barrier in mucosal protection. *Biochim. Biophys. Acta, Gen. Subj.* **2015**, *1850*, 236−252.

(7) Crick, F. Central dogma of molecular biology. *Nature* **1970**, *227*, 561−563.

(8) Zaher, H. S.; Green, R. Fidelity at the molecular level: lessons from protein synthesis. *Cell* **2009**, *136*, 746−762.

(9) Kunkel, T. A.; Bebenek, K. DNA replication fidelity. *Annu. Rev. Biochem.* **2000**, *69*, 497−529.

(10) Reynolds, N. M.; Lazazzera, B. A.; Ibba, M. Cellular mechanisms that control mistranslation. *Nat. Rev. Microbiol.* **2010**, *8*, 849−856.

(11) Poulton, J. M.; ten Wolde, P. R.; Ouldridge, T. E. Nonequilibrium correlations in minimal dynamical models of polymer copying. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 1946−1951.

(12) Johnson, K. A. Conformational coupling in DNA polymerase fidelity. *Annu. Rev. Biochem.* **1993**, *62*, 685−713.

(13) Krüger, H.; Bauer, J.; Rübner, J. Ein modell zur beschreibung reversibler copolymerisationen. *Makromol. Chem.* **1987**, *188*, 2163−2175.

(14) Gaspard, P.; Andrieux, D. Kinetics and thermodynamics of first-order Markov chain copolymerization. *J. Chem. Phys.* **2014**, *141*, 044908.

(15) Shu, Y.-G.; Song, Y.-S.; Ou-Yang, Z.-C.; Li, M. A general theory of kinetics and thermodynamics of steady-state copolymerization. *J. Phys.: Condens. Matter* **2015**, *27*, 235105.

(16) Coote, M. L.; Davis, T. P.; Radom, L. *Models for Free-Radical Copolymerization Propagation Kinetics*; ACS Publications, 2000.

(17) Song, Y.-S.; Shu, Y.-G.; Zhou, X.; Ou-Yang, Z.-C.; Li, M. Proofreading of DNA polymerase: a new kinetic model with higher-order terminal effects. *J. Phys.: Condens. Matter* **2017**, *29*, 025101.

(18) Fu, W.; Li, Q.; Song, Y.; Shu, Y.; Ouyang, Z.; Li, M. Theoretical analysis of RNA polymerase fidelity: a steady-state copolymerization approach. *Commun. Theor. Phys.* **2022**, *74*, 015601.

(19) Andrieux, D.; Gaspard, P. Nonequilibrium generation of information in copolymerization processes. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9516−9521.

(20) Gaspard, P. Growth and dissolution of macromolecular Markov chains. *J. Stat. Phys.* **2016**, *164*, 17−48.

(21) Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems*; Wiley: New York, 1975; Vol. *115*.

(22) Engel, P. C. *Enzyme Kinetics: The Steady-State Approach*; Springer: New York, NY, 2013.

(23) Bel, G.; Munsky, B.; Nemenman, I. The simplicity of completion time distributions for common complex biochemical processes. *Phys. Biol.* **2010**, *7*, 016003.

(24) 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.

(25) de Oliveira, L. P.; Hudebine, D.; Guillaume, D.; Verstraete, J. J. A review of kinetic modeling methodologies for complex processes. *Oil Gas Sci. Technol.* **2016**, *71*, 45.

(26) Laidler, K. J.; Keith, J. *Chemical Kinetics*; McGraw-Hill: New York, 1965; Vol. *2*.

(27) Cao, J. Michaelis-Menten Equation and Detailed Balance in Enzymatic Networks. *J. Phys. Chem. B* **2011**, *115*, 5493−5498.

(28) 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.

(29) Ninio, J. Kinetic amplification of enzyme discrimination. *Biochimie* **1975**, *57*, 587−595.

(30) 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.

(31) Mallory, J. D.; Igoshin, O. A.; Kolomeisky, A. B. Do we understand the mechanisms used by biological systems to correct their errors? *J. Phys. Chem. B* **2020**, *124*, 9289−9296.

(32) 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.

(33) Mallory, J. D.; Mallory, X. F.; Kolomeisky, A. B.; Igoshin, O. A. Theoretical analysis reveals the cost and benefit of proofreading in coronavirus genome replication. *J. Phys. Chem. Lett.* **2021**, *12*, 2691−2698.

(34) Midha, T.; Mallory, J. D.; Kolomeisky, A. B.; Igoshin, O. A. Synergy among Pausing, Intrinsic Proofreading, and Accessory Proteins Results in Optimal Transcription Speed and Tolerable Accuracy. *J. Phys. Chem. Lett.* **2023**, *14*, 3422−3429.

(35) Huang, W. Y.; Alvarez, S.; Kondo, Y.; Kuriyan, J.; Groves, J. T. Relating cellular signaling timescales to single-molecule kinetics: A first-passage time analysis of Ras activation by SOS. *Proc. Natl. Acad. Sci. U.S.A.* **2021**, *118*, No. e2103598118.

(36) Le Novère, N.; Endler, L. In *In Silico Systems Biology*; Schneider, M. V., Ed.; Humana Press: Totowa, NJ, 2013; pp 147−167.
(37) Makarov, D. E. *Single Molecule Science: Physical Principles and Models*; CRC Press, 2015.
(38) 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.
(39) Chanda, M. *Introduction to Polymer Science and Chemistry: A Problem-Solving Approach*; CRC press, 2006.
(40) Gillespie, D. T. Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem.* **1977**, *81*, 2340−2361.
(41) Sartori, P.; Pigolotti, S. Kinetic versus energetic discrimination in biological copying. *Phys. Rev. Lett.* **2013**, *110*, 188101.

https://doi.org/10.1021/acs.jpcb.4c02173
J. Phys. Chem. B XXXX, XXX, XXX−XXX