PHYSICAL REVIEW E **93**, 056401 (2016)

Comment on "Ribosome utilizes the minimum free energy changes to achieve the highest decoding rate and fidelity"

Yonatan Savir*
Department of Physiology, Biophysics and Systems Biology, Faculty of Medicine, Technion, Haifa, 31096, Israel

Tsvi Tlusty
Center for Soft and Living Matter, Institute for Basic Research, UNIST, Ulsan, Korea;
Department of Physics, Ulsan National Institute for Science and Technology, Ulsan, Korea;
Simons Center for Systems Biology, Institute for Advanced Study, Einstein Drive, Princeton NJ 08540, USA;
and Simons Center for Systems Biology, Institute for Advanced Study, Princeton, New Jersey 08540, USA
(Received 16 September 2015; published 13 May 2016)

We examined [Y. Savir and T. Tlusty, Cell **153**, 471 (2013)] the decoding performance of tRNA by the ribosome. For this purpose, we specified the kinetics of tRNA decoding and the corresponding energy landscape, from which we calculated the steady-state decoding rate R_C. Following our work, Xie reexamined [P. Xie, Phys. Rev. E **92**, 022716 (2015)] the energy landscape of tRNA decoding. His analysis relies on an alternative expression for R_C, while claiming that the expression we use is missing some terms. In this Comment we rederive in detail our expression for the steady-state decoding rate R_C, show they hold, explain why the alternative expression for R_C is inaccurate, and discuss the underlying intuition.

DOI: 10.1103/PhysRevE.93.056401

In [1] we introduced a framework to analyze the performance of information flow in biological systems based on molecular recognition in the context of tRNA decoding by the ribosome. We derived a general condition on the structure of the optimal reaction landscape, which is insensitive to the choice of the fitness function. The decoding pathway is a multistep process, which includes an initial selection step, separated by an irreversible energy utilization step from a kinetic proofreading step [2–11]. In [1] we focused on the initial selection step. The author of [12] utilized our framework in attempting to analyze also the proofreading step. The analysis of [12] relies on an alternative expression for the steady-state tRNA decoding rate per free ribosome per free tRNA, R_C, while claiming that in [1] "some terms in the equation of the decoding rate were missed." Quite the opposite, we show here in detail that our result for R_C in [1] holds, while we highlight the basic misassumptions that lead to the inaccurate expression in [12].

Derivation of the steady-state decoding rate. Figure 1 shows the kinetic reaction pathway of the initial selection of the aminoacyl-tRNA by the ribosome [4]. The decoding rate is the GTP activation rate, which is also the production rate of the state S_4 in Fig. 1, $v_{\rm dec} = k_3[S_3] = d[S_4]/dt$. The decoding rate $v_{\rm dec}$ is the number of GTP activation events per unit time per unit volume. The influx of a free ribosome S_1 and free aminoacyl-tRNA t into the pathway is given by $v_{\rm in} = k_1[S_1][t]$. Part of this influx is propagating through the pathway and part of it is rejected at the initial binding stage $v_{\rm rej} = k_{-1}[S_2]$. In steady state, where the net flux vanishes and the intermediate concentrations are constant with respect to time, the decoding rate is given by solving the equations for the concentrations of the states along the initial selection pathway $[S_2]$, and $[S_3]$:

$$\begin{aligned}\frac{d}{dt}[S_2] &= k_1[S_1][t] - (k_{-1}+k_2)[S_2] + k_{-2}[S_3] \\ &= v_{\rm in} - v_{\rm rej} - k_2[S_2] + k_{-2}[S_3] = 0, \\ \frac{d}{dt}[S_3] &= k_2[S_2] - (k_{-2}+k_3)[S_3] = 0. \end{aligned} \tag{1}$$

The solution of the linear equations (1) is straightforward, by eliminating $[S_2]$, which yields

$$v_{\rm dec} = k_3[S_3] = [S_1][t]\frac{k_1k_2k_3}{k_2k_3 + k_{-1}(k_{-2}+k_3)}. \tag{2}$$

As expected, the steady-state decoding rate is simply the difference between the influx and the rejection flux influx $v_{\rm dec} = v_{\rm in} - v_{\rm rej}$.

The decoding rate per *normalized influx,* that is, the decoding rate per *free* ribosome concentration per *free* tRNA concentration, is obtained from Eq. (2):

$$R_C = \frac{v_{\rm dec}}{(v_{\rm in}/k_1)} = \frac{v_{\rm dec}}{[t][S_1]} = \frac{k_1k_2k_3}{k_2k_3 + k_{-1}(k_{-2}+k_3)}, \tag{3}$$

which is Eq. (5) in [1] and Eq. (C3) in [12], without any missing terms. Note that in our notation, as in other works [5–7,11], the measured kinetic constant k_1 has dimensions of $\mu{\rm M}^{-1}\,{\rm s}^{-1}$, whereas the author of [12] denotes by the same symbol the product $k_1^{\rm Xie} = k_1[t]$.

The origin of the inaccuracies in Ref. [12]. In [12], Xie claimed that there are missing terms in our expression for the decoding rate [Eq. (3)] and suggested an alternative expression [Eq. (C1) in [12]]. As explained in the following, these claims originate from two critical inaccuracies in the analysis of [12]: (a) misinterpretation of our results and mixing experimental quantities of different dimensions and (b) misassumption regarding the total conservation of codons.

*yoni.savir@technion.ac.il

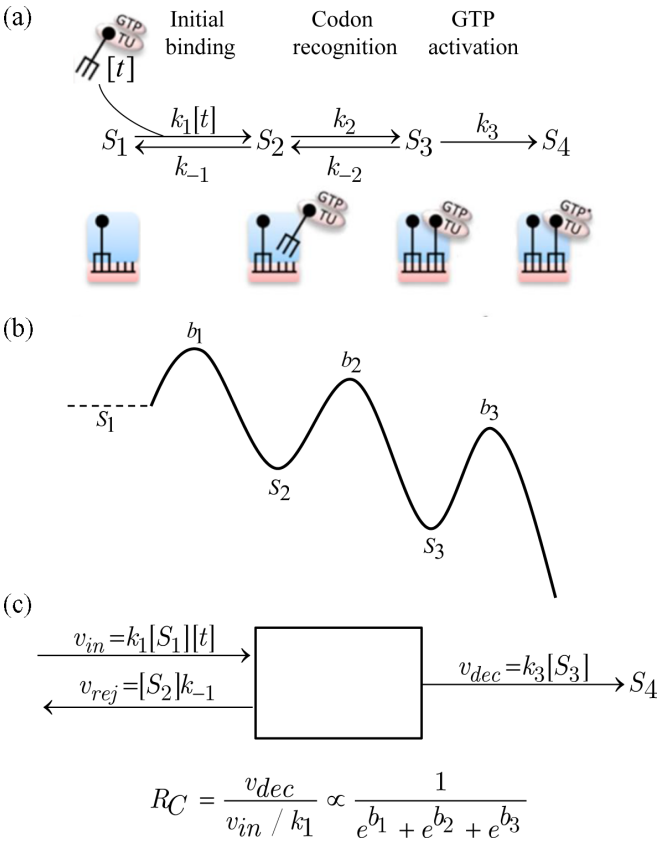

FIG. 1. (a) Basic decoding schema. Free aminoacyl-tRNA, $[t]$, bounds to a free ribosome, S_1, and undergoes a series of modifications. (b) Corresponding free energy landscape of the reaction. (c) Incoming and outgoing fluxes in the reaction. At steady state, the net flux is zero and the normalized decoding rate R_C depends only on the free energy barriers.

In [12], Xie chose to express the decoding rate as a function of the sum of the concentrations of the first three stages,

$$[S_{123}] = [S_1] + [S_2] + [S_3]. \tag{4}$$

By combining assumption (4) with Eq. (1), the decoding rate takes the form

$$v_{\rm dec} = [S_{123}]\frac{k_1[t]k_2k_3}{k_1[t](k_{-2}+k_2+k_3)+k_2k_3+k_{-1}(k_{-2}+k_3)} \tag{5}$$

and the normalized rate per the sum of ribosomes *in any of the first three stages* is

$$R_{\rm Xie} = \frac{v_{\rm dec}}{[S_{123}]} = \frac{k_1[t]k_2k_3}{k_1[t](k_{-2}+k_2+k_3)+k_2k_3+k_{-1}(k_{-2}+k_3)}. \tag{6}$$

Note that, while k_1 in our notation is the measured rate in units of $\mu{\rm M}^{-1}\,{\rm s}^{-1}$, Xie [12] lumps the concentration dependence of the initial binding step into one kinetic parameter $k_1^{\rm Xie} = k_1[t]$, which yields Eq. (C1) in [12].

$$R_{\rm Xie} = \frac{v_{\rm dec}}{[S_{123}]} = \frac{k_1^{\rm Xie}k_2k_3}{k_1^{\rm Xie}(k_{-2}+k_2+k_3)+k_2k_3+k_{-1}(k_{-2}+k_3)}. \tag{7}$$

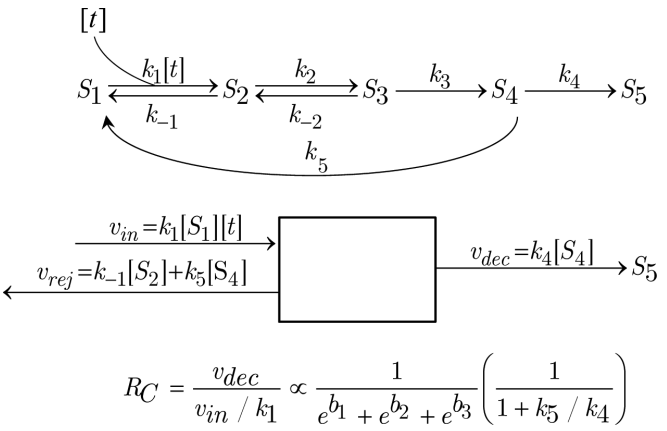

FIG. 2. Adding a rejection step to the reaction in Fig. 1. The decoding rate is the same as the rate without the rejection step, multiplied by the probability to propagate forward out of S_4, which depends on the ratio of k_5/k_4 and thus only on the energy barriers.

The practice of using the total enzyme concentration is common, for example, in deriving the Michaelis-Menten equation, since the assumption of a constant total enzyme concentration is convenient in fitting experimental data (see the Appendix) [13]. However, the sum of the first three stages $[S_{123}]$ is *not* equal to the total concentration of tRNA or ribosomes. There are more tRNAs and ribosomes further downstream the decoding reaction. Thus, the decoding rate $R_{\rm Xie}$ suggested in [12] is the rate per free tRNA per the total sum of concentrations of the ribosome in the first three stages and has no clear biological relevance. In addition, note that $R_{\rm Xie}$ has an explicit dependence on the concentration of free tRNA, $[t]$, which is of course different for cognate or near-cognate tRNAs, thereby rendering the alternative expression unsuitable for the analysis of decoding performance.

The claims repeated a few times in [12] that there are missing terms in our derivation are the result of confusing basic biochemical concepts such as the difference between total and free concentrations. Furthermore, in Appendix C in [12], Xie compares the correct expression [Eq. (2)] to the Eq. (7), while confusing two quantities of different dimensions *as the same*. In our notation [1], k_1 is a second-order kinetic parameter without concentration dependence, while the k_1 of [12] is a first-order kinetic parameter.

Intuition for the decoding rate energy landscape dependence. The decoding rate in steady state (2) can be rewritten as

$$v_{\rm dec} \propto v_{\rm in}\frac{1}{1+e^{b_2-b_1}+e^{b_3-b_1}}. \tag{8}$$

That is, given the influx of the tRNAs and free ribosomes, the decoding rate depends *solely* on the barriers of the energy landscape. The rate $v_{\rm dec}$ has no dependence on the free energies of the intermediate states s_2 and s_3, as changing them has the *same* effect on both the forward and backward fluxes from the intermediate state. Our normalized decoding rate R_C [Eq. (3)] is therefore the decoding rate per material influx which, in steady state, depends only on the energy barriers [Eq. (6) in [1]

and Eq. (C4) in [12]]

$$R_C \propto \frac{1}{e^{b_1} + e^{b_2} + e^{b_3}}. \tag{9}$$

In contrast, the alternative expression [Eqs. (47) and (48) in [12]] results from the erroneous expression for the rate R_{Xie}. We note that adding a rejection step as shown in Fig. 2 does not change the fact that the decoding rate depends only on the barriers of the reaction.

To conclude, we rederived in detail and proved the validity of our expression in [1] for the steady-state decoding rate R_C.

Acknowledgments. T.T. acknowledges support by the Institute for Basic Science, Project No. IBS-R020-D1. Y.S. is a David and Inez Myers Career Advancement Chair in Life Sciences fellow.

Appendix. We review the derivation of Michalis-Menten kinetics [13], $E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_2} E + P$. In steady state ($d[ES]/dt = 0$), the production rate $R = d[P]/dt = k_2[ES]$ is given by

$$R = k_2 \frac{k_1}{k_{-1} + k_2}[E][S] = \frac{k_{\mathrm{cat}}}{K_M}[E][S], \tag{A1}$$

where $[E]$ denotes free enzyme concentration, $[S]$ denotes the free substrate concentration, $k_{\mathrm{cat}} = k_2$, and $K_{\mathrm{M}} = (k_{-1} + k_2)/k_1$. Taking into account the total concentration of the enzyme $E_T = [E] + [ES]$, Eq. (A1) becomes

$$R = E_T k_{\mathrm{cat}} \frac{[S]}{[S] + K_M} = v_{\max} \frac{[S]}{[S] + K_M}, \tag{A2}$$

where $v_{\max} = E_T k_{\mathrm{cat}}$.

[1] Y. Savir and T. Tlusty, The ribosome as an optimal decoder: A lesson in molecular recognition, Cell **153**, 471 (2013).
[2] J. J. Hopfield, Kinetic proofreading: A new mechanism for reducing errors in biosynthetic processes requiring high specificity, Proc. Natl. Acad. Sci. USA **71**, 4135 (1974).
[3] J. Ninio, Kinetic amplification of enzyme discrimination, Biochimie **57**, 587 (1975).
[4] M. V. Rodnina and W. Wintermeyer, Fidelity of aminoacyl-tRNA selection on the ribosome: Kinetic and structural mechanisms, Annu. Rev. Biochem. **70**, 415 (2001).
[5] S. C. Blanchard *et al.*, tRNA selection and kinetic proofreading in translation, Nat. Struct. Mol. Biol. **11**, 1008 (2004).
[6] K. B. Gromadski and M. V. Rodnina, Kinetic determinants of high-fidelity tRNA discrimination on the ribosome, Mol. Cell **13**, 191 (2004).
[7] M. V. Rodnina *et al.*, Recognition and selection of tRNA in translation, FEBS Lett. **579**, 938 (2005).
[8] J. Ninio, Multiple stages in codon-anticodon recognition: Double-trigger mechanisms and geometric constraints, Biochimie **88**, 963 (2006).
[9] T. H. Lee *et al.*, The role of fluctuations in tRNA selection by the ribosome, Proc. Natl. Acad. Sci. USA **104**, 13661 (2007).
[10] R. A. Marshall *et al.*, Translation at the single-molecule level, Annu. Rev. Biochem. **77**, 177 (2008).
[11] H. S. Zaher and R. Green, Fidelity at the molecular level: Lessons from protein synthesis, Cell **136**, 746 (2009).
[12] P. Xie, Ribosome utilizes the minimum free energy changes to achieve the highest decoding rate and fidelity, Phys. Rev. E **92**, 022716 (2015).
[13] A. R. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman, San Francisco, 1998).