With the leisure of time, kinetic proofreading does reliable ligand discrimination after all

Fangzhou Xiao[1*] and Vahe Galstyan[2†]

[1]Department of Physics, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093, USA
[2]AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands

In a recent *PNAS* publication, Kirby and Zilman argued that when stochasticity is taken into account, having more steps in kinetic proofreading generally does not improve the discrimination of different receptor-binding ligands based on the counts of signaling molecules produced [1]. However, their conclusion is based on a major technical error that, once corrected, contradicts their main conclusion. Also, throughout their analysis, the authors made the implicit assumption that discrimination time is fixed. We argue below that, for a fuller understanding, kinetic proofreading for ligand discrimination should be studied in the context of speed-accuracy tradeoffs.

Once a straight-up mistake is corrected, results in Kirby and Zilman actually show that discrimination performance improves with more proofreading steps. As stated in their main text, to calculate false activation probability in Fig. 4C, Kirby and Zilman chose the signal level for 10% activation chance under the *strongly* binding ligand as the activation threshold. However, in their code generating Fig. 4C, they mistakenly calculated the activation threshold under the *weakly* binding ligand. While Fig. 4C in Kirby and Zilman suggests that the false activation probability increases with more proofreading steps, the corrected code for exactly the same model and parameters generates Fig. 1b, showing the opposite. This behavior become intuitive if we look at the distributions of signaling molecules (Fig. 1a), where it is clear that the probability for the weakly binding ligand signal (purple) to reach the activation threshold (black dashed line) decreases with N. Although the probability in Fig 1b is unmeaningfully low due to their choice of threshold, discrimination improving with N does hold for activation thresholds up to $\approx 80\%$ true activation chance, beyond which the false activation probability may decrease with N (Fig. 1c).

Depending on time, discrimination performance increases or decreases with more proofreading steps. It is well-known that proofreading exhibits speed-accuracy tradeoff [2]. Kirby and Zilman, however, do not discuss proofreading in this context; instead, they fixed the time to produce signaling molecules before the cell is asked to discriminate. While assuming fixed time can have discrimination improve with N (Fig. 1b), this assumption is not always warranted, since the discrimination time could naturally vary with N due to feedback [3] or signaling threshold mechanisms [4].

We therefore study proofreading as a function of both N and the discrimination time. If we allow time to vary, e.g. to keep the signal concentration fixed for downstream activity, then we recover the canonical behavior where false activation probability uniformly increases with more proofreading steps (Figs. 1f and 1g). More generally, investigating the speed-accuracy tradeoff (Fig. 1h) uncovers two distinct regimes: when time is short, more proofreading steps always give better discrimination; when time is long, more proofreading steps lead to worse discrimination. This highlights that discrimination performance of proofreading schemes should be discussed in the context of speed-accuracy tradeoff for a fuller understanding.

* fxiao@ucsd.edu
† v.galstyan@amolf.nl

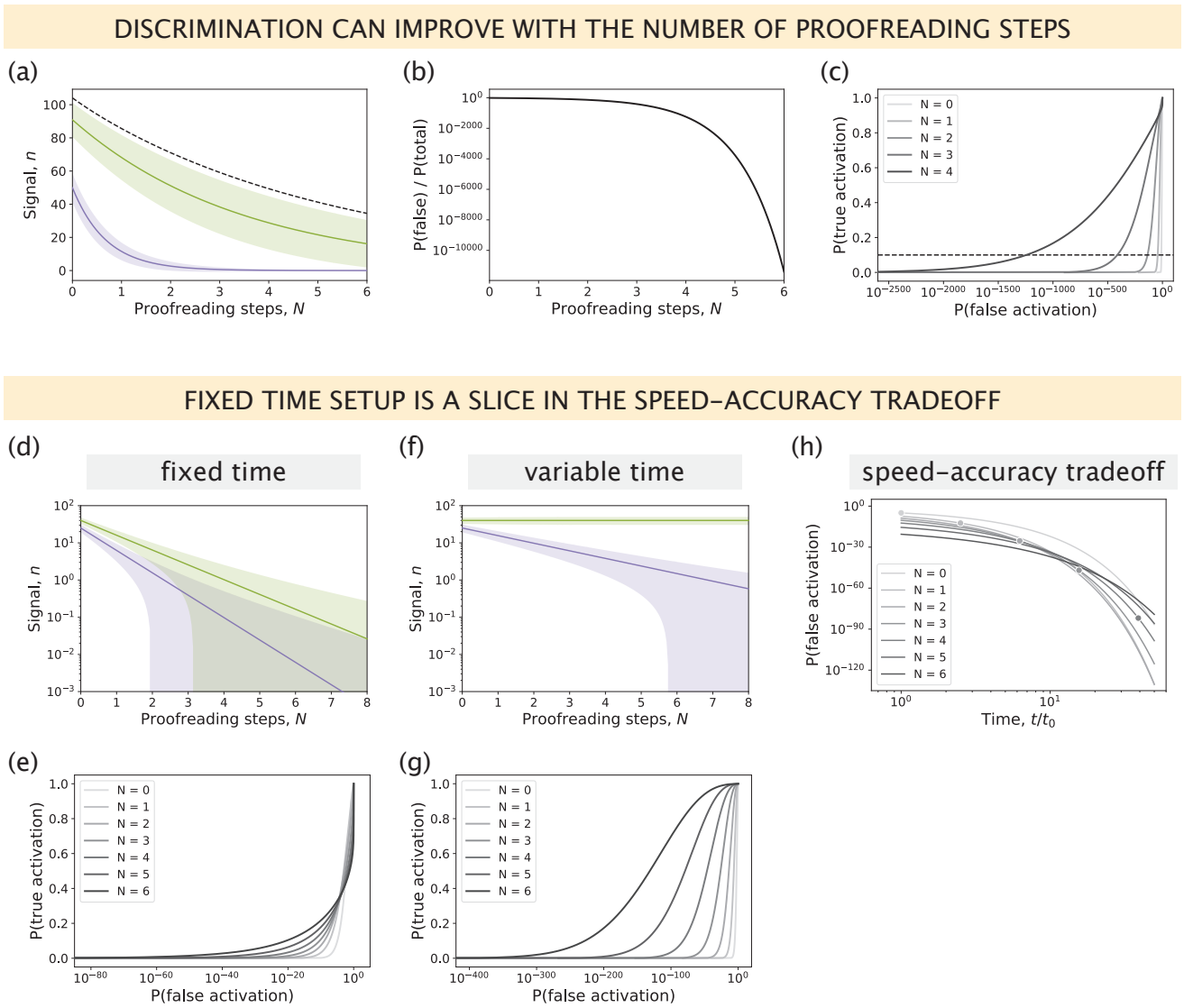

Figure 1: **Discrimination performance can exhibit diverse responses to varying the number of proofreading steps. (a)** Signal arising from strongly binding (green) and weakly binding (purple) ligand binding (mean $\pm$ standard deviation) as a function of the number of proofreading steps (N) in a fixed time setting. The dashed line indicates the activation threshold n^* defined via $P(n(k_{off,2}) > n^*) = 0.1$. Parameters used are the same as in Fig. 4C of Kirby and Zilman: $k_{on}c/k_p = 1$, $k_p t = 100$, $k_{off,1}/k_p = 1$, $k_{off,2}/k_p = 0.1$, $k_f/k_p = 0.3$ (weaker proofreading strength). **(b)** False activation probability over total activation probability (sum of true and false activation probabilities) as a function of N for the setup in panel (a). This is the corrected version of Fig. 4C of Kirby and Zilman for the case of weaker proofreading strength ($k_f = 0.3$); clearly, discrimination improves with N. We note that the probabilities are calculated in exactly the way Kirby and Zilman intended, using a Gaussian approximation. **(c)** True vs. false activation probability curves (ROC curves) for different discrete choices of N. The dashed line at $y = 0.1$ corresponds to the threshold in panel (a). **(d)** Signals as a function of N in the fixed time setting, the corrected version of Fig. 2B of Kirby and Zilman with the same parameters ($k_{on}c/k_p = 1$, $k_f/k_p = 1$, $k_p t = 100$, $k_{off,1}/k_p = 3$, and $k_{off,2}/k_p = 1.5$). Their code has a mistake in the order of parameter substitution. **(e)** ROC curves for different N corresponding to panel (d). **(f)** Signals as a function of N where the discrimination time t_N scales with N as $t_N = t_0(1+k_{off,2}/k_f)^N$ with $k_p t_0 = 100$. All other parameters are the same as in panel (d). **(g)** ROC curves for different N corresponding to panel (f). Clearly, proofreading improves with N. **(h)** Speed-accuracy tradeoff curves for different choices of N, with speed captured by discrimination time and accuracy captured by false activation probability (computed for a threshold true activation probability of 0.1). Dots correspond to the variable time setting in panel (f).

References

1. Kirby, D. & Zilman, A. Proofreading does not result in more reliable ligand discrimination in receptor signaling due to its inherent stochasticity. *Proceedings of the National Academy of Sciences* **120,** e2212795120 (2023).
2. Murugan, A., Huse, D. A. & Leibler, S. Speed, dissipation, and error in kinetic proofreading. *Proceedings of the National Academy of Sciences* **109,** 12034–12039 (2012).
3. François, P., Voisinne, G., Siggia, E. D., Altan-Bonnet, G. & Vergassola, M. Phenotypic model for early T-cell activation displaying sensitivity, specificity, and antagonism. *Proceedings of the National Academy of Sciences* **110,** E888–E897 (2013).
4. Morgan, J. & Lindsay, A. E. Modulation of antigen discrimination by duration of immune contacts in a kinetic proofreading model of T cell activation with extreme statistics. *PLOS Computational Biology* **19,** 1–17 (Aug. 2023).