Kumar, Adhikari, and Dua Reply: In the preceding Comment [1], Jeong *et al.* show, using renewal theory, that the inverse of the mean waiting time between turnovers of N enzymes obeys the Michaelis-Menten equation, which they claim is in "direct contradiction" with our result in [2]. Further, they impute to our work the claim that "the mean catalytic rate of a system of enzymes at mesoscopic concentration does not obey the Michaelis-Menten equation." As we show below, using both numerical simulation and exact analytics, the "direct contradiction" obtained in [1] is due to an erroneous comparison of the statistics of turnovers obtained in two incompatible limits. Further, as our work does not address the delicate question of the definition of the mean catalytic rate for stochastic enzyme kinetics, but only focuses on the statistics of turnovers, their imputation is unwarranted.

We note, first, that in our study, *all* enzymes are chosen to be in their free state at $t = 0$, the mesoscopic analog of the initial condition that, in the macroscopic limit, yields the Michaelis-Menten equation (MME). Revisiting the numerical simulations in [2] we show, in the top panel of Fig. 1, the convergence of the waiting time distributions to a limiting form with increasing turnover number p. For turnover numbers much greater than a critical value $p^\star$, the waiting times are identically distributed. In addition, correlations between waiting times, though appreciable for $p \ll p^\star$, become negligible for $p \gg p^\star$. Thus, the critical turnover number p^* marks the crossover from a transient regime $p \ll p^*$, where nonrenewal statistics obtains, to a steady-state regime $p \gg p^*$, where the renewal property is asymptotically recovered *even* for a mesoscopic number of enzymes.

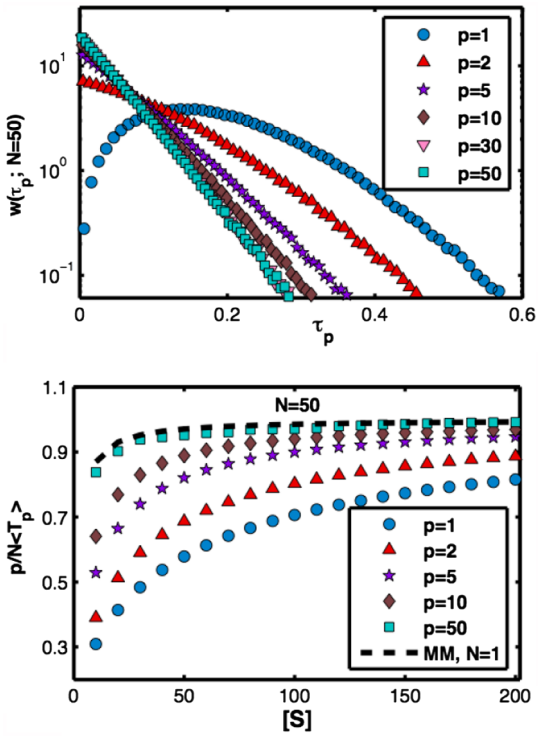

FIG. 1. Waiting time distributions from numerical simulations (top) and scaled inverse mean turnovers times from exact analytical results (bottom), with varying turnover number p, in the limit of many turnovers. MM indicates the Michaelis-Menten equation.

To support the above numerical result analytically, we examine the $p \to \infty$ limit of the exact analytical result, Eq. (6) of [2], for the distribution $w(T_p)$ of the pth turnover time T_p. This involves computing the p derivatives of the generating function, obtained exactly from the chemical master equation (CME) in Eq. (4) of [2]. From this exact, if somewhat tedious, calculation we show in the bottom panel of Fig. 1, the variation of the inverse of the scaled mean turnover time, $(p/N\langle T_p\rangle)$, with the amount of substrate. We find that for steady-states $p \gg p^\star$, the scaled inverse mean turnover time asymptotes to that for a single enzyme which, as established in [2], obeys the MME. For the transient regime $p \ll p^\star$, the limit studied in [2], substantial departures from the MME remain.

This motivates the definition $v(N) = p/\langle T_p(N)\rangle$ of the enzymatic velocity in a mesoscopic ensemble in terms of the mean turnover times that is valid for *all* p, both transient and steady state. It yields a *steady-state* enzymatic velocity obeying the MME in the large turnover limit,

$$v_{ss}(N) = \lim_{p\to\infty} \frac{p}{\langle T_p(N)\rangle} = \frac{k_a k_2 N}{k_a + k_b} \tag{1}$$

in agreement with the renewal theory result in [1]. Therefore, the "direct contradiction" claimed in [1] resolves itself as an erroneous comparison between a waiting time computed in the steady-state limit $p \gg p^\star$ with another computed in the transient regime $p \ll p^\star$. To emphasise, while [1] *assumes* without justification a steady-state limit with renewal property, we *demonstrate* it numerically and *derive* it analytically, from the CME. The renewal approach fails for transient states $p \ll p^\star$, where correlations are non-negligible, cannot describe the crossover from the transient to the steady-state $p \gg p^\star$, nor estimate the critical turnover time. All of these can be accomplished in complete generality with the CME [2] which provides, thus, a powerful tool for deriving turnover statistics from kinetic mechanisms and for studying the correlated enzymatic turnovers that have been the focus of much recent experiment [3].

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