Stochastic simulations data for figure 1 and the phase diagram construction for defining monotonic and non-monotonic regimes of the velocity as a function of $k_{off}$

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**Abstract**

We have compared our theoretical expressions of the normalized reaction velocities with that of simulation data points generated when the substrate fluctuations are present and absent, for the reaction schemes represented in Figure 1 Singh and Chaudhury, 2019 in the general monotonic as well as the conditional non-monotonic limit. We have also constructed the phase diagrams for the schemes given in Figure 1 Singh and Chaudhury, 2019 separating different regimes of the monotonic and the non-monotonic behaviors observed in the reaction rate.

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1. Data

We have compared the normalized reaction velocities from theory and simulations for the reaction schemes represented in Fig. 1 [1] at a fixed value of substrate concentration (shown in Fig. 1 of this article) as a function of $k_{off}$ and $k_{off}^{(1)}$, respectively. We have also performed a comparative study between the normalized rates from theory and simulations for those schemes [1] at a given $[S]$ under the non-monotonic limit (represented in Fig. 2 of this article) as a function of $k_{off}$ and $k_{off}^{(1)}$, respectively.
2. Experimental design, materials, and methods

2.1. Comparison of the theoretical expressions for the normalized velocity with the stochastic simulations for a general parameter set

Fig. 1. (a) Comparison of the normalized reaction velocity from theory and simulations for the reaction scheme represented in (a) Fig. 1(a) [1] at $[S] = 2$. The reaction rate constants are $k_{on} = 1$, $\alpha = 1$, $\beta = 2$, and $k_{cat} = 5$. (b) Fig. 1(b) [1] at $[S] = 10$, as a function of $k_{off}$ and $k_{off}^{(1)}$, respectively. The reaction rate constants are $k_{on} = 1$, $\alpha = 1$, $\beta_1 = 2$, $\beta_2 = 4$ and $k_{cat} = 5$. The red solid lines represent the stochastic simulations carried out taking $[S]$ as a variable (filled red squares are the generated data points), the filled green circles represent the simulation points at constant $[S]$ and the black solid lines represent Eq. 11 and Eq. A.9 [1] in (a) and (b), respectively.

2.2. Comparison of the theoretical expressions for the normalized velocity with the stochastic simulations in the non-monotonic limit

Fig. 2. Comparison of the normalized reaction velocity in the non-monotonic limit for the reaction scheme represented in (a) Fig. 1(a) [1] at $[S] = 15$ with $k_{cat} > \frac{10 \cdot (\alpha - \beta) - (\alpha - \beta)^2}{(\alpha - \beta)^2}$, $\alpha > \beta$. The set of reaction rate constants are $k_{on} = 1$, $\alpha = 3$, $\beta = 1$ and $k_{cat} = 20$. (b) Fig. 1(b) [1] at $[S] = 15$ with $k_{cat} > \frac{10 \cdot (\alpha - \beta) - (\alpha - \beta)^2}{(\alpha - \beta)^2}$, $\alpha > \beta_2$, as a function of $k_{off}$ and $k_{off}^{(1)}$, respectively. The set of reaction rate constants in the plot are $k_{on} = 1$, $\alpha = 4$, $\beta_1 = 2$, $\beta_2 = 1$ and $k_{cat} = 25$. The filled red squares represent the stochastic simulation data points obtained when $[S]$ is taken as a constant and the black solid lines represent Eq. 11 and Eq. A.9 [1] in (a) and (b), respectively.
2.3. Mathematical procedure followed for the phase diagram construction

Rearranging eq 10 [1] in terms of the unbinding rate constant \(k_{\text{off}}\) we get

\[
v_{1P} = \left( \pi_1 + \pi_2 k_{\text{off}} + \frac{\pi_3}{k_{\text{off}} + \pi_4} \right)^{-1}
\]

where,

\[
\pi_1 = 2 \left( \frac{1}{k_{\text{cat}}} + \frac{1}{k_{\text{on}} |S|} \right), \quad \pi_2 = \frac{2}{k_{\text{cat}} k_{\text{on}} |S|}, \quad \pi_3 = 1 - \frac{\beta}{\alpha} - \frac{2 \beta}{k_{\text{on}} |S|} \quad \text{and} \quad \pi_4 = \beta \left( 2 + \frac{k_{\text{on}} |S|}{\alpha} \right).
\]

Differentiating \(v_{1P}\) (eq (1)) with respect to \(k_{\text{off}}\) and putting the limit \(k_{\text{off}} \to 0\), we get

\[
\left( \frac{dv_{1P}}{dk_{\text{off}}} \right)_{k_{\text{off}} \to 0} = \frac{\pi_1}{(\pi_4)^2} - \frac{\pi_2}{\left( \pi_1 + \frac{\pi_3}{\pi_4} \right)^2}
\]

For the general set of kinetic parameters, we find that \(\left( \frac{dv_{1P}}{dk_{\text{off}}} \right)_{k_{\text{off}} \to 0} < 0\) as \(\frac{\pi_1}{(\pi_4)^2} < \pi_2\). Thus, the reaction velocity is a continuously decreasing function of \(k_{\text{off}}\) as shown in Fig. 2(a).

We solve eq 13 [1] and obtain a particular range of \(|S|\) in which non-monotonicity in the velocity is observed and the parameter values satisfy the limits mentioned in eq 14a and eq 14b [1]. As shown in Fig. 2(b), the velocity derivative with respect to \(k_{\text{off}}\) \(\left( \frac{dv_{1P}}{dk_{\text{off}}} \right)_{k_{\text{off}} \to 0}\) increases initially and then decreases \(\left( \frac{\pi_1}{(\pi_4)^2} < \pi_2 \right)\). To construct the phase diagram (Fig. 3(a)) that can separate different monotonic and non-monotonic regions of velocity, we plot \(\frac{\pi_3}{(\pi_4)^2}\) as a function of \(\pi_2\). We define the non-monotonicity index (\(\emptyset\)) as

\[
\emptyset = \frac{\pi_3}{(\pi_4)^2} - \pi_2
\]
If $\varnothing > 1$, it represents the region in which the velocity will show a non-monotonic (non-MM) behavior. As discussed in the main text, this will be observed only in a certain range of the substrate concentration with some particular choice of parameter values.

$0 < \varnothing \leq -1$ represents the regime in which the velocity will show a monotonic (non-MM) behavior for any given set of kinetic parameters. When $\varnothing < -1$ it represents the region in which the velocity attains the MM form and decreases monotonically.

For Fig. 1(b), rearranging eq 12 [1] in terms of the unbinding rate constant $k_{\text{off}}^{(1)}$ we get

$$v_{2p} = \left( \frac{\pi_5 + \pi_2 k_{\text{off}}^{(1)} + \pi_6}{k_{\text{off}}^{(1)} + \pi_7} \right)^{-1}$$

(4)

where,

$$\pi_2 = \frac{2}{k_{\text{cat}} k_{\text{on}}, \pi_5 = \frac{\beta_1 + \beta_2}{\beta_2 k_{\text{on}}} + \frac{2}{k_{\text{on}} |S|}, \pi_6 = \beta_1 \left( \frac{1}{\alpha} + \frac{1}{\beta_2} - \frac{2}{k_{\text{on}} |S|} \right) \text{ and } \pi_7 = \beta_1 \left( 2 + \frac{k_{\text{on}} |S|}{\alpha} \right)$$

Differentiating eq (4) with respect to $k_{\text{off}}^{(1)}$ and putting the limit $k_{\text{off}}^{(1)} \to 0$, we get

$$\frac{d v_{2p}}{d k_{\text{off}}^{(1)}} = \frac{\pi_6}{\pi_7} - \frac{\pi_2}{(\pi_5 + \pi_6)^2}$$

(5)

The non-monotonic velocity will only be observed if $\frac{\pi_6}{\pi_7} > \pi_2$. Otherwise, the reaction velocity will show a monotonic behavior when studied as a function of $k_{\text{off}}^{(1)}$.

We construct the phase diagram as shown in Fig. 3(b) separating different regions of velocity and plot $\frac{\pi_6}{\pi_7}$ as a function of $\pi_2$. For the scheme represented in Fig. 1(b), the non-monotonicity index is defined as

$$\varnothing = \frac{\frac{\pi_6}{\pi_7}}{\pi_2}$$

(6)

As described earlier, $\varnothing > 1$, $0 < \varnothing \leq -1$ and $\varnothing < -1$ represent regime with non-monotonic (non-MM), monotonic (non-MM) and monotonic MM behavior, respectively. We have constructed a phase diagram which divides different regions of the reaction rate (depicted in Fig. 3 of this article).

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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