

# SUPPLEMENTARY MATERIAL

MATHEMATICS-1964681

## Kinetic analysis of one-step suicide substrate inactivation of an enzyme-catalyzed ping-pong reaction with one substrate undergoing disproportionation

Ismael Gutiérrez-Fernández, Ouardia Bendou, Nara Bueno-Ramos, Emilio L. Marcos-Barbero, Rosa Morcuende and Juan B. Arellano

*Note about section 3.3.1 of the main body of manuscript*

A close-form solution for the time dependent variation of [A] in Equation 53 can be represented as follows:

$$[A] = (K_M^E + K_M^F) \times W \left[ \frac{[A]_0}{(K_M^E + K_M^F)} \text{Exp} \left( \frac{-\frac{2k_c k_f [E]_0 t}{(k_c + k_f)} + [A]_0}{(K_M^E + K_M^F)} \right) \right], \quad (S1)$$

where  $K_M^E$  and  $K_M^F$  are the Michaelis-Menten constants for the first and second stages of a ping-pong reaction in which the substrate undergoes disproportionation. More information about the symbols is given in Table 1.

*Note about section 3.3.2 of the main body of manuscript*

The two alternative approaches to solve analytically Reactions 8 and 9 presented in Case B of the section 3.3.2 are explained below.

### 1. Steady-state approximation for E and F

The non-linear ODE system [Equations (60) and (61)] of the time-dependent variation of the concentration for the participating compounds of Reactions (54) and (55) can easily be solved if the steady-state is invoked for [E] and, consequently, for [F] (*i.e.*,  $[E]' \approx 0$  and  $[F]' \approx 0$ ). If this is the case, the analytical solutions for [A], [E] and [F] with initial values for  $[A] = [A]_0$  and  $[E] = [E]_0$  are as follows:

$$[A] = [A]_0 e^{-\frac{2k_d k_a [E]_0 t}{k_a + k_d}}, \quad (S2)$$

$$[E] = \frac{k_d}{k_a + k_d} [E]_0, \quad (S3)$$

$$[F] = \frac{k_a}{k_a + k_d} [E]_0. \quad (S4)$$

Of interest is also the fact that the integrated solution for [A] [Equation (S1)] could also have been derived from the integration of Equation (50) if  $k_c$  and  $k_f$  were assumed to approach infinity. However, unless the condition  $[A]_0 \gg [E]_0$  still remains, the approximate integrated solution for [A] is not completely satisfactory. This can clearly be observed in Figure S1, in which the values for  $k_a$  and  $k_d$  were simply exchanged and the values for  $[A]_0$  and  $[E]_0$  were of similar order. The numerical and analytical solutions showed a better overlapping for conditions in which  $k_a \leq k_d$  when compared to  $k_a > k_d$ . The enzyme states E and F reach more rapidly steady-state when  $k_a \leq k_d$  and the match between the numerical and analytical solutions of the time-dependent variation of the concentration and rate for the

substrate and (the sum of) products was maintained through a broader domain of time (Fig S1A, C and E). In contrast, when  $k_a > k_d$ , a more noticeable mismatch between the numerical and analytical solutions for the compounds were observed, even beyond the time at which E and F were already considered to be in steady-state (Fig S1B, D and F).

## 2. Non-steady-state approximation for E and F

At time around  $t = 0$ , when  $[E] \approx [E]_0$ , the first terms of the power expansion series of the enzyme-dependent function of  $[E]'$  [Equation (66)] were obtained and only the partial sum of the first two polynomials of grade  $n=1$  and  $n=2$  were evaluated to find approximate solutions:

$$[E]_{n=1}' = -k_a [E]_0 [A]_0 - [k_a [E]_0 + (k_a + k_d) [A]_0] ([E] - [E]_0) + \dots, \quad (S5)$$

$$[E]_{n=2}' = -k_a [E]_0 [A]_0 - [k_a [E]_0 + (k_a + k_d) [A]_0] ([E] - [E]_0) - k_a ([E] - [E]_0)^2 + \dots \quad (S6)$$

The approximate integrated solutions for Equations (S5) and (S6) were as follows:

$$[E]_{n=1} = \frac{[E]_0 \left[ k_a [E]_0 + (k_a e^{-k_{ad1}t} + k_d) [A]_0 \right]}{k_{ad1}}, \quad (S7)$$

$$[E]_{n=2} = \frac{1}{2k_a} \left[ k_{ad1} + k_{ad2} \text{Tanh} \left[ \frac{1}{2} k_{ad2} t + \text{ArcTanh} \left( \frac{k_{ad1}}{k_{ad2}} \right) \right] \right], \quad (S8)$$

where  $k_{ad1}$ ,  $k_{ad1}^-$  and  $k_{ad2}$  were constants that depended on  $[A]_0$ ,  $[E]_0$  and the rate constants  $k_a$  and  $k_d$  as shown below:

$$k_{ad1} = k_a [E]_0 + (k_a + k_d) [A]_0, \quad (S9)$$

$$k_{ad1}^- = k_a [E]_0 - (k_a + k_d) [A]_0, \quad (S10)$$

$$k_{ad2} = \left[ k_a^2 [E]_0^2 - 2k_a (k_a - k_d) [E]_0 [A]_0 + (k_a + k_d)^2 [A]_0^2 \right]^{1/2}. \quad (S11)$$

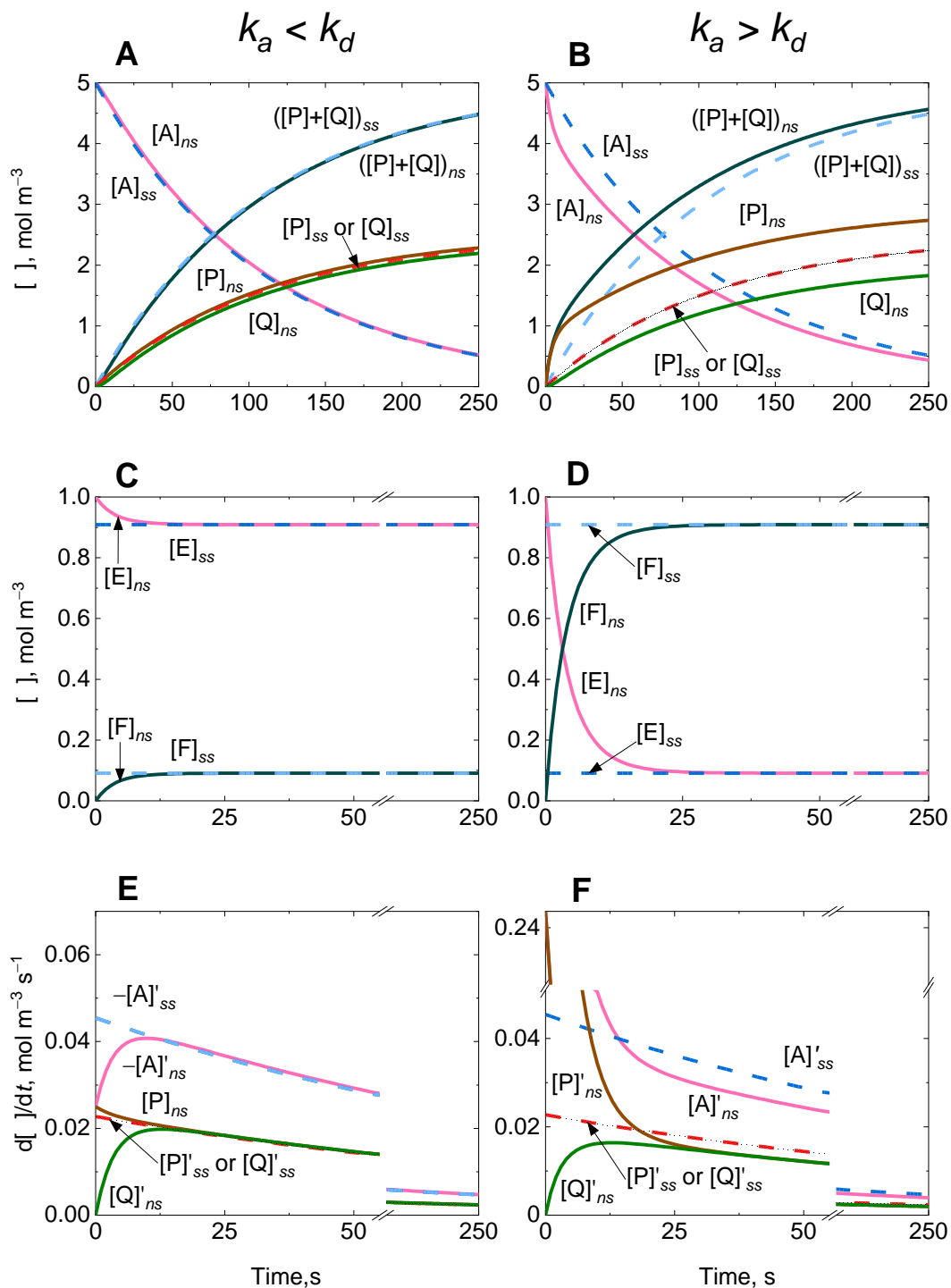
After the respective substitution of  $[E]_{n=1}$  and  $[E]_{n=2}$  for  $[E]$  in Equation (62), the approximate integrate solutions for  $[A]_{n=1}$  and  $[A]_{n=2}$  were derived:

$$[A]_{n=1} = [A]_0 + \frac{(k_a^2 - k_d^2) [E]_0 \left( \frac{k_a [E]_0 + (k_a e^{-k_{ad1}t} + k_d) [A]_0}{k_{ad1}} - 1 \right) + 2k_a k_d [E]_0 \ln \left[ \frac{k_a [E]_0 + (k_a + k_d) e^{-k_{ad1}t} [A]_0}{k_{ad1}} \right]}{(k_a + k_d)^2}, \quad (S12)$$

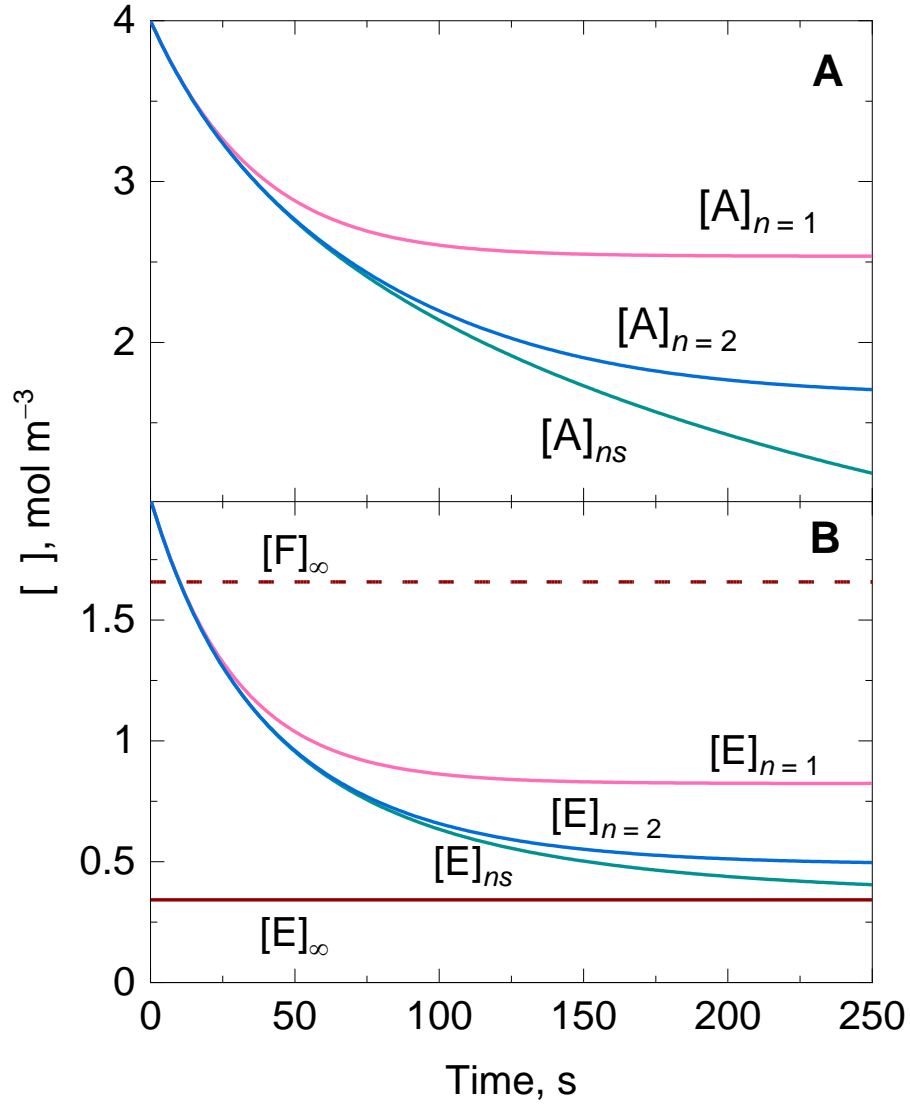
$$[A]_{n=2} = [A]_0 - \frac{(k_a - k_d) \left( k_{ad1} - k_{ad2} \text{Tanh} \left[ \frac{k_{ad2} t}{2} + \text{ArcTanh} \left( \frac{k_{ad1}}{k_{ad2}} \right) \right] \right)}{2k_a (k_a + k_d)} + \frac{2k_a k_d [E]_0}{(k_a + k_d)^2} \ln \left[ \frac{k_a k_{ad1}^- - k_d k_{ad1} + k_{ad2} (k_a + k_d) \text{Tanh} \left[ \frac{k_{ad2} t}{2} + \text{ArcTanh} \left( \frac{k_{ad1}}{k_{ad2}} \right) \right]}{2k_a^2 [E]_0} \right]. \quad (S13)$$

The approximate analytical solutions for  $[A]$  and  $[E]$  together with their respective numerical solutions are shown in Figure 2S. The graphs illustrate how the matches between the numerical and approximate analytical solutions for  $[A]$  and  $[E]$  improve around  $t=0$  under non-steady-state conditions as the grade of the polynomial increases. However, the approximate analytical solutions for  $[A]$  and  $[E]$  have the inconvenience of growing complexity as  $n$  increases. The mismatch between the

numerical and approximate integrated solutions for [A] and [E] was more prominent as the reaction progressed and [A] became exhausted.



**Figure S1.** Representative numerical (dashed lines, subscript *ns*) and analytical (solid lines, subscript *ss*) solutions of the time-dependent variation of (A–D) the concentration for the substrate A, the products P and Q, and the two active enzyme states E and F and (E, F) the reaction rate for A, P and Q of an enzyme-catalyzed ping-pong reaction. The substrate A follows disproportionation and the accumulation of the intermediate substrate-enzyme complexes EA and FA is negligible. For the analytical solution, E and F were assumed to be in steady-state. Initial conditions:  $[A]_0 = 5 \text{ mol m}^{-3}$ ,  $[E]_0 = 1 \text{ mol m}^{-3}$ , and (A, C and E)  $k_a = 5 \times 10^{-3} \text{ m}^3(\text{mol s})^{-1}$  and  $k_d = 5 \times 10^{-2} \text{ m}^3(\text{mol s})^{-1}$  or (B, D and F)  $k_a = 5 \times 10^{-2} \text{ m}^3(\text{mol s})^{-1}$  and  $k_d = 5 \times 10^{-3} \text{ m}^3(\text{mol s})^{-1}$ .



**Figure S2.** Representative numerically (subscript  $ns$ ) and analytically (subscript  $n=1$  or  $2$ ) integrated solutions of the time-dependent variation of the concentration for (A) the substrate A and (B) the two active enzyme states E and F of an enzyme catalyzed ping-pong reaction under non-steady-state conditions. The substrate A undergoes disproportionation and the substrate-enzyme complexes AE and AF do not accumulate. The numerical solutions ( $ns$ ) for  $[A]$  and  $[E]$  are shown together with two approximate integrated solutions around  $t=0$  obtained using the first terms ( $n=1$  or  $2$ ) of the power expansion series of the enzyme-dependent function of  $[E]'$ . Horizontal solid and dashed lines are the limit values of the analytical solution for  $[E]$  and  $[F]$  (*i.e.*,  $[E]_{\infty}$  and  $[F]_{\infty}$ , Equation 52) when the times goes forward and  $[A]$  becomes exhausted. Initial conditions:  $[A]_0 = 4 \text{ mol m}^{-3}$ ,  $[E]_0 = 2 \text{ mol m}^{-3}$ ,  $k_a = 5 \times 10^{-3} \text{ m}^3(\text{mol s})^{-1}$  and  $k_d = 10^{-3} \text{ m}^3(\text{mol s})^{-1}$ .