Supplementary Material

Single-enzyme approach predicts natural emergence of inhibitor-activator duality

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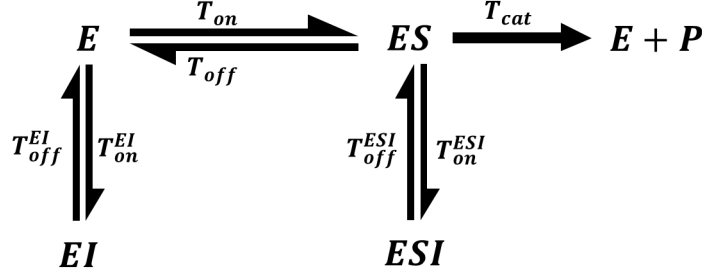

Figure S1: A generic scheme for mixed inhibition at the single enzyme level. Transition rates were replaced by generally distributed transition times.

A generic scheme describing enzymatic catalysis under mixed inhibition is illustrated in Fig. S1. We now analyze this scheme to derive an expression for the enzymatic turnover rate, and will later on show that results in the main text follow as special cases. Consider a single enzyme that is found initially in its free state (E), and further consider the *random* time it takes the enzyme to reach state $(E + P)$ having started there. We refer to this time as the turnover time of the reaction, denote it by T_{turn}, and further note that it is given by

$$T_{turn} \equiv F_E = W_E + \begin{cases} F_{ES} & \text{if } T_{on} < T_{on}^{EI} \\ \\ F_{EI} & \text{if } T_{on} > T_{on}^{EI}. \end{cases} \tag{1}$$

Here, F_E, F_{ES}, and F_{EI} denote the random times taken to reach the $(E + P)$ state, for the first time, having started at states (E), (ES), and (EI) correspondingly. In addition, T_{on} is the random time taken to bind a substrate molecule, T_{on}^{EI} is the random time taken to bind an inhibitor molecule, and $W_E = min(T_{on}, T_{on}^{EI})$ is the random time spent at state (E) prior to its departure. What determines the nature of the transition from state (E) to one of its two neighboring states is whether binding of a substrate molecule preceded that of an inhibitor molecule, $T_{on} < T_{on}^{EI}$, or vice versa $T_{on} > T_{on}^{EI}$. In the former case $T_{turn} = W_E + F_{ES}$ while in the latter we have $T_{turn} = W_E + F_{EI}$. We thus see that T_{turn} cannot be determined in isolation as it also requires knowledge of F_{ES} and F_{EI}.

To proceed, let us first note that F_{EI} is related to other times in the problem via the following relation

$$F_{EI} = W_{EI} + T_{turn} = T_{off}^{EI} + T_{turn}, \tag{2}$$

where we noted that $W_{EI} = T_{off}^{EI}$ since the random time spent in state EI prior to its departure is simply the time it takes for the inhibitor to unbind the enzyme. In addition, we see that

$$F_{ES} = W_{ES} + \begin{cases} 0 & \text{if } T_{cat} < T_{off}, T_{on}^{ESI} \\ \\ T_{turn} & \text{if } T_{off} < T_{cat}, T_{on}^{ESI} \\ \\ F_{ESI} & \text{if } T_{on}^{ESI} < T_{off}, T_{cat} \end{cases} \tag{3}$$

where T_{cat} is the random time taken to complete the catalytic step, and T_{off} and T_{on}^{ESI} are respectively the random times taken for the substrate to unbind and the inhibitor to bind the (ES) state. Finally, we note that

$$F_{ESI} = W_{ESI} + F_{ES} = T_{off}^{ESI} + F_{ES} \,, \tag{4}$$

where we have again noted that $W_{ESI} = T_{off}^{ESI}$ since the random time spent in state ESI is simply the time taken for the inhibitor to unbind this state.

Equations (1-4) completely specify the set of relations between the random times T_{turn}, F_{EI}, F_{ES} and F_{ESI}. Taking expectations we then have a set of four equations

$$\langle T_{turn} \rangle = \langle W_E \rangle + Pr\left(T_{on} < T_{on}^{EI}\right) \langle F_{ES} \rangle + Pr\left(T_{on} > T_{on}^{EI}\right) \langle F_{EI} \rangle \,,$$

$$\langle F_{EI} \rangle = \langle T_{off}^{EI} \rangle + \langle T_{turn} \rangle \,,$$

$$\langle F_{ES} \rangle = \langle W_{ES} \rangle + Pr\left(T_{off} < T_{cat} \,, T_{on}^{ESI}\right) \langle T_{turn} \rangle + Pr\left(T_{on}^{ESI} < T_{off} \,, T_{cat}\right) \langle F_{ESI} \rangle \,, \tag{5}$$

$$\langle F_{ESI} \rangle = \langle T_{off}^{ESI} \rangle + \langle F_{ES} \rangle \,,$$

for the unknowns $\langle T_{turn} \rangle, \langle F_{EI} \rangle, \langle F_{ES} \rangle$ and $\langle F_{ESI} \rangle$, and solving for $\langle T_{turn} \rangle$ gives

$$\langle T_{turn} \rangle = \begin{cases} \dfrac{\left(1 - Pr\left(T_{on}^{ESI} < T_{off}, T_{cat}\right)\right)\langle W_E \rangle}{Pr(T_{cat} < T_{off}, T_{on}^{ESI})Pr(T_{on} < T_{on}^{EI})} + \dfrac{\langle W_{ES} \rangle}{Pr(T_{cat} < T_{off}, T_{on}^{ESI})} \\[1.5em] + \dfrac{\left(1 - Pr\left(T_{on} < T_{on}^{EI}\right)\right)\left(1 - Pr\left(T_{on}^{ESI} < T_{off}, T_{cat}\right)\right)\langle T_{off}^{EI} \rangle}{Pr(T_{cat} < T_{off}, T_{on}^{ESI})Pr(T_{on} < T_{on}^{EI})} \\[1.5em] + \dfrac{Pr\left(T_{on}^{ESI} < T_{off}, T_{cat}\right)\langle T_{off}^{ESI} \rangle}{Pr(T_{cat} < T_{off}, T_{on}^{ESI})} \,. \end{cases} \tag{6}$$

The right hand side of Eq. (6) depends on certain probabilities and expectations values but these can all be computed given information on the underlying transitions times which govern the problem. Indeed, with $f_X(t)$ denoting the probability density of a random variable X and $\bar{F}_X(t) = Pr(X > t) = 1 - \int_0^t f_X(t')dt'$ as the complimentary cumulative distribution function of this random variable, we have

$$Pr\left(T_{on}^{ESI} < T_{off}, T_{cat}\right) = \int_0^\infty f_{T_{on}^{ESI}}(t)\, \bar{F}_{T_{off}}(t)\, \bar{F}_{T_{cat}}(t)\, dt \,,$$

$$Pr\left(T_{cat} < T_{off}, T_{on}^{ESI}\right) = \int_0^\infty f_{T_{cat}}(t)\, \bar{F}_{T_{off}}(t)\, \bar{F}_{T_{on}^{ESI}}(t)\, dt \,, \tag{7}$$

$$Pr\left(T_{on} < T_{on}^{EI}\right) = \int_0^\infty f_{T_{on}}(t)\, \bar{F}_{T_{on}^{EI}}(t)\, dt \,.$$

The mean times $\langle W_E \rangle$ and $\langle W_{ES} \rangle$ can also be written in a similar way exploiting the fact that the time spent at a state is a minimum over the occurrence times of competing processes governing the departure from this state. For example, W_E is nothing but a minimum over T_{on} and T_{on}^{EI}. We thus have $\bar{F}_{W_E}(t) = Pr(W_E > t) = \bar{F}_{T_{on}}(t)\, \bar{F}_{T_{on}^{EI}}(t)$ and hence

$$\langle W_E \rangle = \int_0^\infty \bar{F}_{W_E}(t)dt = \int_0^\infty \bar{F}_{T_{on}}(t)\, \bar{F}_{T_{on}^{EI}}(t)\, dt \,. \tag{8}$$

Similarly, we find that

$$\langle W_{ES} \rangle = \int_0^\infty \bar{F}_{W_{ES}}(t)dt = \int_0^\infty \bar{F}_{T_{off}}(t)\, \bar{F}_{T_{on}^{ESI}}(t)\, \bar{F}_{T_{cat}}(t)dt \,. \tag{9}$$

1.1 Simplified expressions for the case of exponential binding times

The result in Eq. (6) can be simplified by taking advantage of the fact that many substrate molecules independently compete for the binding of the same enzyme. And so, while the stochastic time characterizing the binding of a single substrate molecule may be complex, the amalgamation of many independent binding attempts will follow Poisson statistics. This in turn means that the binding time T_{on} comes from an exponential distribution with density

$$f_{T_{on}}(t) = k_{on}[S] e^{-k_{on}[S]t} . \tag{10}$$

Using the same rational for the binding time of the inhibitor to the enzyme, T_{on}^{EI}, and to the enzyme substrate complex, T_{on}^{ESI}, gives

$$f_{T_{on}^{EI}}(t) = k_{on}^{EI}[I] e^{-k_{on}^{EI}[I]t} ,$$

$$f_{T_{on}^{ESI}}(t) = k_{on}^{ESI}[I] e^{-k_{on}^{ESI}[I]t} , \tag{11}$$

for the probability density functions of these random variables.

1.1.1 Turnover in the absence of inhibition

In the absence of inhibitor molecules the probability of binding one is zero, and we thus have $Pr\left(T_{on}^{ESI} < T_{off}, T_{cat}\right) = 0$, $Pr\left(T_{on} < T_{on}^{EI}\right) = 1$, and $Pr\left(T_{cat} < T_{on}^{ESI}, T_{off}\right) = Pr\left(T_{cat} < T_{off}\right)$. It then follows that the mean time spent in state E is simply the time it takes a substrate to bind the enzyme, $\langle W_E \rangle = \langle T_{on} \rangle$, and Eq. (6) becomes

$$\langle T_{turn} \rangle = \frac{\langle T_{on} \rangle + \langle min\left(T_{cat}, T_{off}\right)\rangle}{Pr\left(T_{cat} < T_{off}\right)} . \tag{12}$$

Equation (10) then implies that $\langle T_{on} \rangle^{-1} = k_{on}[S]$ and rearmament of (12) gives

$$\langle T_{turn} \rangle = \frac{1}{Pr\left(T_{cat} < T_{off}\right) k_{on}[S]} + \frac{\langle min\left(T_{cat}, T_{off}\right)\rangle}{Pr\left(T_{cat} < T_{off}\right)} . \tag{13}$$

Comparing the result in Eq. (13) to the classical Michaelis–Menten equation $\langle T_{turn} \rangle = \frac{K_m}{v_{max}} \frac{1}{[S]} + \frac{1}{v_{max}}$, we identify the constants

$$v_{max} = \frac{Pr(T_{cat} < T_{off})}{\langle min(T_{cat}, T_{off})\rangle} = \frac{\int_0^\infty f_{T_{cat}}(t)\bar{F}_{T_{off}}(t)dt}{\int_0^\infty \bar{F}_{T_{cat}}(t)\bar{F}_{T_{off}}(t)dt} ,$$

$$K_m = \frac{1}{k_{on}\langle min(T_{cat}, T_{off})\rangle} = \frac{1}{k_{on}\int_0^\infty \bar{F}_{T_{cat}}(t)\bar{F}_{T_{off}}(t)dt} , \tag{14}$$

and note that in order to get these expressions we did not make any assumptions on the distributions of the catalysis time T_{cat} and unbinding time T_{off}.

1.1.2 Turnover with inhibition

To progress analysis in the case where inhibitors are present, we first note that Eq. (14) asserts that

$$\int_0^\infty f_{T_{cat}}(t)\bar{F}_{T_{off}}(t)dt = \frac{v_{max}}{K_m k_{on}} , \tag{15}$$

and

$$\int_0^\infty \bar{F}_{T_{cat}}(t)\bar{F}_{T_{off}}(t)dt = \frac{1}{K_m k_{on}} . \tag{16}$$

Using this fact, we define two normalized probability density functions $f_M(t)$ and $f_P(t)$

$$f_M(t) = K_m k_{on}\bar{F}_{T_{cat}}(t)\bar{F}_{T_{off}}(t) ,$$

$$f_P(t) = \frac{K_m k_{on}}{v_{max}} f_{T_{cat}}(t)\bar{F}_{T_{off}} , \tag{17}$$

and their corresponding Laplace transforms $\tilde{f}_M(s)$ and $\tilde{f}_P(s)$

$$\tilde{f}_M(s) = \int_0^\infty e^{-st} K_m k_{on} \bar{F}_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) \, dt \,,$$

$$\tilde{f}_P(s) = \int_0^\infty e^{-st} \frac{K_m k_{on}}{v_{max}} f_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) dt \,. \tag{18}$$

Using these definitions, and by use of Eq. (11), Eq. (7) can be simplified and written in the following form

$$Pr\left(T_{on}^{ESI} < T_{off}, T_{cat}\right) = \frac{k_{on}^{ESI}[I]}{K_m k_{on}} \int_0^\infty e^{-k_{on}^{ESI}[I]t} f_M(t) \, dt = \frac{k_{on}^{ESI}[I]}{K_m k_{on}} \tilde{f}_M\left(k_{on}^{ESI}[I]\right) \,,$$

$$Pr\left(T_{cat} < T_{off}, T_{on}^{ESI}\right) = \frac{v_{max}}{K_m k_{on}} \int_0^\infty e^{-k_{on}^{ESI}[I]t} f_P(t) \, dt = \frac{v_{max}}{K_m k_{on}} \tilde{f}_P\left(k_{on}^{ESI}[I]\right) \,, \tag{19}$$

$$Pr\left(T_{on} < T_{on}^{EI}\right) = \frac{k_{on}[S]}{k_{on}[S] + k_{on}^{EI}[I]} \,.$$

Doing the same for Eqs. (8-9) we find

$$\langle W_E \rangle = \frac{1}{k_{on}[S] + k_{on}^{EI}[I]} \,, \tag{20}$$

$$\langle W_{ES} \rangle = \frac{1}{K_m k_{on}} \int_0^\infty e^{-k_{on}^{ESI}[I]t} f_M(t) \, dt = \frac{1}{K_m k_{on}} \tilde{f}_M\left(k_{on}^{ESI}[I]\right) \,. \tag{21}$$

Substituting Eq. (19-21) back into Eq. (6) the latter can be simplified to give

$$\langle T_{turn} \rangle = \frac{K_m \left(1 + k_{on}^{EI} \left\langle T_{off}^{EI} \right\rangle [I]\right)}{v_{max}} \frac{A([I])}{[S]} + \frac{\left(1 + k_{on}^{ESI} \left\langle T_{off}^{ESI} \right\rangle [I]\right) B([I])}{v_{max}} \,, \tag{22}$$

where

$$A([I]) = \frac{1 - \frac{k_{on}^{ESI}[I]}{K_m k_{on}} \tilde{f}_M\left(k_{on}^{ESI}[I]\right)}{\tilde{f}_P\left(k_{on}^{ESI}[I]\right)} \,, \tag{23}$$

and

$$B([I]) = \frac{\tilde{f}_M\left(k_{on}^{ESI}[I]\right)}{\tilde{f}_P\left(k_{on}^{ESI}[I]\right)} \,. \tag{24}$$

Equations (23-24) coincide with Eqs. (M3-M4) in the methods section of the main text. Recalling that $\langle T_{turn} \rangle \equiv 1/k_{turn}$, $K_{EI} = \left(k_{on}^{EI} \left\langle T_{off}^{EI} \right\rangle\right)^{-1}$, and $K_{ESI} = \left(k_{on}^{ESI} \left\langle T_{off}^{ESI} \right\rangle\right)^{-1}$, we see that Eq. (22) coincides with Eq. (5) in the main text, and we will now show that all of the results in the main text could be derived from it.

2 Proof that Eq. (5) in the main text reduces to Eq. (1) when all transition times are exponentially distributed

When all the transition times in Fig. S1 are exponentially distributed Eqs. (10-11) remain valid, and in addition we have

$$f_{T_{cat}}(t) = k_{cat}e^{-k_{cat}t} \,,$$

$$f_{T_{off}}(t) = k_{off}e^{-k_{off}t} \,,$$

$$f_{T_{off}^{EI}}(t) = k_{off}^{EI}e^{-k_{off}^{EI}t} \,,$$

$$f_{T_{off}^{ESI}}(t) = k_{off}^{ESI}e^{-k_{off}^{ESI}t} \,,$$

$$(25)$$

for the probability density functions of $T_{cat}, T_{off}, T_{off}^{EI}$, and T_{off}^{ESI}. We then find that

$$Pr\left(T_{cat} < T_{off}\right) = \int_0^\infty f_{T_{cat}}(t)\,\bar{F}_{T_{off}}(t)\,dt = \int_0^\infty k_{cat}e^{-k_{cat}t}e^{-k_{off}t}dt = \frac{k_{cat}}{k_{off}+k_{cat}} \,, \quad (26)$$

and

$$\langle min\left(T_{cat}, T_{off}\right)\rangle = \int_0^\infty \bar{F}_{T_{cat}}(t)\,\bar{F}_{T_{off}}(t)\,dt = \int_0^\infty e^{-k_{cat}t}e^{-k_{off}t}dt = \frac{1}{k_{off}+k_{cat}} \,, \quad (27)$$

from which it follows that

$$v_{max} = \frac{Pr(T_{cat}<T_{off})}{\langle min(T_{cat},T_{off})\rangle} = k_{cat} \,,$$

$$K_m = \frac{1}{k_{on}\langle min(T_{cat},T_{off})\rangle} = \frac{k_{off}+k_{cat}}{k_{on}} \,.$$

$$(28)$$

In addition, from Eqs. (17-18) we now have

$$\tilde{f}_M(k_{on}^{ESI}[I]) = \frac{k_{off}+k_{cat}}{k_{on}}k_{on}\int_0^\infty e^{-k_{on}^{ESI}[I]t}e^{-k_{cat}t}e^{-k_{off}t}dt = \frac{k_{off}+k_{cat}}{k_{off}+k_{cat}+k_{on}^{ESI}[I]} \,,$$

$$\tilde{f}_P(k_{on}^{ESI}[I]) = \frac{\frac{k_{off}+k_{cat}}{k_{on}}k_{on}}{k_{cat}}\int_0^\infty e^{-k_{on}^{ESI}[I]t}k_{cat}e^{-k_{cat}t}e^{-k_{off}t}dt = \frac{k_{off}+k_{cat}}{k_{off}+k_{cat}+k_{on}^{ESI}[I]} \,.$$

$$(29)$$

Substituting the above back into Eq. (23-24) we conclude that

$$A\left([I]\right) = \frac{k_{off}+k_{cat}+k_{on}^{ESI}[I]}{k_{off}+k_{cat}}\left[1 - \frac{k_{on}^{ESI}[I]}{k_{off}+k_{cat}}\cdot\frac{k_{off}+k_{cat}}{k_{off}+k_{cat}+k_{on}^{ESI}[I]}\right] = 1 \,, \quad (30)$$

and

$$B\left([I]\right) = 1 \,. \quad (31)$$

Equation (25) moreover asserts that $k_{off}^{EI} = 1/\left\langle T_{off}^{EI}\right\rangle$ and $k_{off}^{ESI} = 1/\left\langle T_{off}^{ESI}\right\rangle$, which in turn means that

$$\langle T_{turn}\rangle = \frac{K_m\left(1 + \frac{k_{on}^{EI}}{k_{off}^{EI}}[I]\right)}{v_{max}}\frac{1}{[S]} + \frac{\left(1 + \frac{k_{on}^{ESI}}{k_{off}^{ESI}}[I]\right)}{v_{max}} \,, \quad (32)$$

and we see that the result in Eq. (32) coincides with Eq. (1) in the main text.

3 Derivation of Eq. (2) in the main text — turnover rate for competitive inhibition with generally distributed transition times

Competitive inhibition can be seen as a special case of mixed inhibition in which the binding rate of the inhibitor to the enzyme-substrate complex is zero ($k_{on}^{ESI} = 0$). Keeping in mind Eqs. (15-17), and the definition Eq. (18), we see that

$$\tilde{f}_M(k_{on}^{ESI}[I] = 0) = \int_0^\infty K_m k_{on} \bar{F}_{T_{cat}}(t) \bar{F}_{T_{off}}(t) \, dt = \int_0^\infty f_M(t) dt = 1 \,,$$

$$\tilde{f}_P(k_{on}^{ESI}[I] = 0) = \int_0^\infty \frac{K_m k_{on}}{v_{max}} f_{T_{cat}}(t) \bar{F}_{T_{off}}(t) dt = \int_0^\infty f_P(t) dt = 1 \,,$$
(33)

and thus have

$$A\left(k_{on}^{ESI}[I] = 0\right) = B\left(k_{on}^{ESI}[I] = 0\right) = 1 \,.$$
(34)

Equation (22) then becomes

$$\langle T_{turn} \rangle = \frac{K_m \left(1 + k_{on}^{EI} \left\langle T_{off}^{EI} \right\rangle [I]\right)}{v_{max}} \frac{1}{[S]} + \frac{1}{v_{max}} \,,$$
(35)

which coincides with Eq. (2) in the main text.

4 Derivation of Eq. (3) in the main text — turnover rate for uncompetitive inhibition with generally distributed transition times

Uncompetitive inhibition can also be seen as a special case of mixed inhibition. Here, the binding rate of the inhibitor to the free enzyme is zero ($k_{on}^{EI} = 0$), and since $A[I]$ and $B[I]$ do not dependent on k_{on}^{EI}, Eq. (22) reduces to

$$\langle T_{turn} \rangle = \frac{K_m}{v_{max}} \frac{A([I])}{[S]} + \frac{\left(1 + k_{on}^{ESI} \left\langle T_{off}^{ESI} \right\rangle [I]\right) B([I])}{v_{max}} \,,$$
(36)

which coincides with Eq. (3) in the main text.

5 The two-state model

The two state model described in the bottom panel of Fig. 4 in the main text is a special case of the more general scheme for uncompetitive inhibition that is described in the top panel of the same figure. Indeed, the two can be shown to coincide by allowing the catalysis time in the general scheme to come from a distribution whose density is

$$f_{T_{cat}}(t) = p k_{cat}^{(1)} e^{-k_{cat}^{(1)} t} + (1-p) k_{cat}^{(2)} e^{-k_{cat}^{(2)} t} \,,$$
(37)

and otherwise taking all transition times to be exponentially distributed with proper rates. To see this, note that in the two state model the ES complex can be found in one of two states: ES_1 or ES_2. However, these states have the same substrate unbinding rate, k_{off}, and inhibitor binding rate, $k_{on}^{ESI}[I]$, and only differ in their catalytic rates which are correspondingly given by $k_{cat}^{(1)}$ and $k_{cat}^{(2)}$. Moreover, a transition from the free enzyme E occurs with rate $k_{on}[S]$ and leads

to ES_1 with probability p and to ES_2 with probability $1-p$, and these states are reached with the exact same probabilities after an inhibitor unbinds, with rate k_{off}^{ESI}, from the ESI complex. We thus see that ES_1 and ES_2 could be effectively merged into a single ES state whose catalysis rate is randomly drawn to be $k_{cat}^{(1)}$ with probability p and $k_{cat}^{(2)}$ with probability $1-p$ every time this state is visited. This asserts that the probability density function of T_{cat} is given by Eq. (37) above, and we further note that from the construction of the two state model it follows that $T_{on}, T_{off}, T_{on}^{ESI}$ and T_{off}^{ESI} are all exponentially distributed with rates $k_{on}[S]$, k_{off}, $k_{on}^{ESI}[I]$ and k_{off}^{ESI}, respectively.

5.1 Derivation of explicit expressions for the functions A([I]) and B([I])

With the above at hand we can derive explicit expressions for $A([I])$ and $B([I])$. We first note that in the case of the two state model Eq. (18) can be written as

$$\tilde{f}_M(k_{on}^{ESI}[I]) = K_m k_{on} \cdot \int_0^\infty e^{-\left(k_{on}^{ESI}[I]+k_{off}\right)t} \bar{F}_{T_{cat}}(t)\,dt = K_m k_{on} \cdot \frac{1-\tilde{f}_{T_{cat}}(k_{off}+k_{on}^{ESI}[I])}{k_{off}+k_{on}^{ESI}[I]} ,$$

$$\tilde{f}_P(k_{on}^{ESI}[I]) = \frac{K_m k_{on}}{v_{max}} \int_0^\infty e^{-\left(k_{on}^{ESI}[I]+k_{off}\right)t} f_{T_{cat}}(t)\,dt = \frac{K_m k_{on}}{v_{max}} \cdot \tilde{f}_{T_{cat}}(k_{off}+k_{on}^{ESI}[I]) , \tag{38}$$

with

$$\tilde{f}_{T_{cat}}(s) = \int_0^\infty e^{-st} f_{T_{cat}}(t)dt = p\frac{k_{cat}^{(1)}}{k_{cat}^{(1)}+s} + (1-p)\frac{k_{cat}^{(2)}}{k_{cat}^{(2)}+s} , \tag{39}$$

standing for the Laplace transform of $f_{T_{cat}}(t)$. Substituting Eq. (38) into Eqs. (23-24) then gives

$$A([I]) = \frac{v_{max}}{(k_{off}+k_{on}^{ESI}[I])K_m k_{on}}\left(\frac{k_{off}}{\tilde{f}_{T_{cat}}(k_{off}+k_{on}^{ESI}[I])} + k_{on}^{ESI}[I]\right) , \tag{40}$$

and

$$B([I]) = \frac{v_{max}}{(k_{off}+k_{on}^{ESI}[I])}\left(\frac{1}{\tilde{f}_{T_{cat}}(k_{off}+k_{on}^{ESI}[I])} - 1\right) . \tag{41}$$

To further proceed, we observe that in the case of the two state model Eq. (14) reduces to

$$v_{max} = \frac{\int_0^\infty f_{T_{cat}}(t)e^{-k_{off}t}dt}{\int_0^\infty \bar{F}_{T_{cat}}(t)e^{-k_{off}t}dt} = \frac{k_{off}\tilde{f}_{T_{cat}}(k_{off})}{1-\tilde{f}_{T_{cat}}(k_{off})} = \frac{k_{off}(pk_{cat}^{(1)}+(1-p)k_{cat}^{(2)})+k_{cat}^{(1)}k_{cat}^{(2)}}{(1-p)k_{cat}^{(1)}+pk_{cat}^{(2)}+k_{off}} ,$$

$$K_m = \frac{1}{k_{on}\int_0^\infty \bar{F}_{T_{cat}}(t)e^{-k_{off}t}dt} = \frac{k_{off}}{k_{on}\left(1-\tilde{f}_{T_{cat}}(k_{off})\right)} = \frac{(k_{cat}^{(1)}+k_{off})(k_{cat}^{(2)}+k_{off})}{k_{on}\left((1-p)k_{cat}^{(1)}+pk_{cat}^{(2)}+k_{off}\right)} . \tag{42}$$

Substituting Eq. (42) into Eqs. (40-41) and making use of Eq. (39) we find

$$A([I]) = 1 - \frac{k_{off}(1-p)p\left(\frac{1}{k_{cat}^{(1)}} - \frac{1}{k_{cat}^{(2)}}\right)^2 k_{on}^{ESI}[I]}{\left(1+\frac{k_{off}}{k_{cat}^{(1)}}\right)\left(1+\frac{k_{off}}{k_{cat}^{(2)}}\right)\left(1+\left(\frac{1-p}{k_{cat}^{(1)}}+\frac{p}{k_{cat}^{(2)}}\right)(k_{off}+k_{on}^{ESI}[I])\right)} , \tag{43}$$

and

$$B([I]) = 1 - \frac{(1-p)p\left(\frac{1}{k_{cat}^{(1)}} - \frac{1}{k_{cat}^{(2)}}\right)^2 k_{on}^{ESI}[I]}{\left(\frac{p}{k_{cat}^{(1)}}+\frac{1-p}{k_{cat}^{(2)}}+\frac{k_{off}}{k_{cat}^{(1)}k_{cat}^{(2)}}\right)\left(1+\left(\frac{1-p}{k_{cat}^{(1)}}+\frac{p}{k_{cat}^{(2)}}\right)(k_{off}+k_{on}^{ESI}[I])\right)} . \tag{44}$$

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It could now be observed that $A([I])$ and $B([I])$ are both monotonically decreasing functions of the inhibitor concentration $[I]$, and that at high inhibitor concentrations these functions approach their asymptotic values

$$A([I] \to \infty) \simeq 1 - \frac{k_{off}(1-p)p \left(\frac{1}{k_{cat}^{(1)}} - \frac{1}{k_{cat}^{(2)}}\right)^2}{\left(1 + \frac{k_{off}}{k_{cat}^{(1)}}\right)\left(1 + \frac{k_{off}}{k_{cat}^{(2)}}\right)\left(\frac{1-p}{k_{cat}^{(1)}} + \frac{p}{k_{cat}^{(2)}}\right)}, \tag{45}$$

and

$$B([I] \to \infty) \simeq 1 - \frac{(1-p)p \left(\frac{1}{k_{cat}^{(1)}} - \frac{1}{k_{cat}^{(2)}}\right)^2}{\left(\frac{p}{k_{cat}^{(1)}} + \frac{1-p}{k_{cat}^{(2)}} + \frac{k_{off}}{k_{cat}^{(1)}k_{cat}^{(2)}}\right)\left(\frac{1-p}{k_{cat}^{(1)}} + \frac{p}{k_{cat}^{(2)}}\right)}. \tag{46}$$

6 Derivation of Eq. (4) in the main text — a condition asserting the emergence of inhibitor-activator duality

To derive a general condition for the emergence of inhibitor-activator duality we start from the expression for the mean turnover time in Eq. (22), and ask when will $\frac{d\langle T_{turn}\rangle}{d[I]}|_{[I]=0} < 0$? In other words, we would like to determine when will an increase in the concentration of the inhibitor, from an initial value of zero, result in a decrease of the mean turnover time, and hence in an increase of the turnover rate. To answer this question we first note that

$$\frac{d\langle T_{turn}\rangle}{d[I]}|_{[I]=0} = \frac{K_m k_{on}^{EI} \langle T_{off}^{EI}\rangle}{v_{max}[S]} + \frac{k_{on}^{ESI} \langle T_{off}^{ESI}\rangle}{v_{max}}$$

$$+ \frac{K_m}{v_{max}[S]} \frac{dA([I])}{d[I]}|_{[I]=0} + \frac{1}{v_{max}} \frac{dB([I])}{d[I]}|_{[I]=0}. \tag{47}$$

Now, since $\tilde{f}_M(s)$ and $\tilde{f}_P(s)$ are the Laplace transforms of the random variables M and P defined by the normalized densities in Eq. (17) we have

$$\tilde{f}_M\left(k_{on}^{ESI}[I]\right) = 1 - k_{on}^{ESI}[I]\langle M\rangle + O([I]^2), \tag{48}$$

and

$$\tilde{f}_P\left(k_{on}^{ESI}[I]\right) = 1 - k_{on}^{ESI}[I]\langle P\rangle + O([I]^2), \tag{49}$$

from which we find that

$$A([I]) = \frac{1 - \frac{k_{on}^{ESI}[I]}{K_m k_{on}} \tilde{f}_M\left(k_{on}^{ESI}[I]\right)}{\tilde{f}_P\left(k_{on}^{ESI}[I]\right)} = 1 + k_{on}^{ESI}[I]\langle P\rangle - \frac{k_{on}^{ESI}[I]}{K_m k_{on}} + O([I]^2), \tag{50}$$

and

$$B([I]) = \frac{\tilde{f}_M\left(k_{on}^{ESI}[I]\right)}{\tilde{f}_P\left(k_{on}^{ESI}[I]\right)} = 1 + k_{on}^{ESI}[I]\langle P\rangle - k_{on}^{ESI}[I]\langle M\rangle + O([I]^2). \tag{51}$$

Plugging these equations back into Eq. (47) we find

$$\frac{d\langle T_{turn}\rangle}{d[I]}|_{[I]=0} = \frac{K_m k_{on}^{EI} \langle T_{off}^{EI}\rangle}{v_{max}[S]} + \frac{k_{on}^{ESI} \langle T_{off}^{ESI}\rangle}{v_{max}}$$

$$\frac{K_m \left(k_{on}^{ESI}\langle P\rangle - \frac{k_{on}^{ESI}}{K_m k_{on}}\right)}{v_{max}[S]} + \frac{k_{on}^{ESI}\langle P\rangle - k_{on}^{ESI}\langle M\rangle}{v_{max}}. \tag{52}$$

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Rearranging we see that $\frac{d\langle T_{turn}\rangle}{d[I]}|_{[I]=0} < 0$ if and only if

$$\frac{K_m k_{on}^{EI} \left\langle T_{off}^{EI} \right\rangle}{k_{on}^{ESI} [S]} + \left(1 + \frac{K_m}{[S]}\right) \langle P \rangle + \left\langle T_{off}^{ESI} \right\rangle - \frac{1}{k_{on}[S]} < \langle M \rangle \ . \tag{53}$$

To make sense of this condition and some more progress we return to the definitions of $\langle P \rangle$ and $\langle M \rangle$ and recall that these are given by

$$\langle M \rangle = K_m k_{on} \int_0^\infty t \cdot \bar{F}_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) \, dt \,, \tag{54}$$

and

$$\langle P \rangle = \frac{K_m k_{on}}{v_{max}} \int_0^\infty t \cdot f_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) dt \,, \tag{55}$$

which in turn means that they could be related to the life time, $W_{ES}^0 = min\left(T_{cat}, T_{off}\right)$, of the enzyme substrate complex in the absence of inhibition. Indeed, recalling the definitions of K_m and v_{max} in Eq. (14) we observe that

$$\begin{aligned} f_P(t) &= \frac{K_m k_{on}}{v_{max}} f_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) = \frac{f_{T_{cat}}(t)\bar{F}_{T_{off}}(t)}{Pr(T_{cat}<T_{off})} \\ &= f_{\{T_{cat}|T_{cat}<T_{off}\}}(t) = f_{\{W_{ES}^0|ES\rightarrow E+P\}}(t) \,, \end{aligned} \tag{56}$$

where $f_{\{W_{ES}^0|ES\rightarrow E+P\}}(t)$ is simply the probability density function of the life time W_{ES}^0 given that the stay in the ES state resulted in product formation (catalysis occurred prior to unbinding). This in turn means that

$$\langle P \rangle = \int_0^\infty t \cdot f_{\{W_{ES}^0|ES\rightarrow E+P\}}(t) dt = \left\langle W_{ES}^0|ES \rightarrow E + P \right\rangle \ . \tag{57}$$

Regarding $\langle M \rangle$, we first note that

$$\begin{aligned} \bar{F}_{W_{ES}^0}(t) &= Pr\left(W_{ES}^0 > t\right) = Pr\left(min\left(T_{cat}, T_{off}\right) > t\right) \\ &= Pr\left(T_{cat} > t\right) Pr\left(T_{off} > t\right) = \bar{F}_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) \,, \end{aligned} \tag{58}$$

i.e., that the life time of the ES state (in the absence of inhibition) is larger than t if and only if neither catalysis nor unbinding occurred by that time. Equation (54) could then be written as

$$\langle M \rangle = K_m k_{on} \int_0^\infty t \cdot \bar{F}_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) \, dt = \frac{\int_0^\infty t \cdot \bar{F}_{T_{cat}}(t) \, \bar{F}_{T_{off}}(t) \, dt}{\langle min\left(T_{cat}, T_{off}\right) \rangle} = \frac{\int_0^\infty t \cdot \bar{F}_{W_{ES}^0}(t) \, dt}{\langle W_{ES}^0 \rangle} \,, \tag{59}$$

where we have once again used the definition of K_m in Eq. (14). The nominator can be worked out using integration by parts and we find

$$\int_0^\infty t \cdot \bar{F}_{W_{ES}^0}(t) \, dt = \frac{t^2}{2} \bar{F}_{W_{ES}^0}(t) \Big|_0^\infty + \frac{1}{2} \int_0^\infty t^2 \cdot f_{W_{ES}^0}(t) dt = \lim_{t\rightarrow\infty} \left\{\frac{1}{2} t^2 \bar{F}_{W_{ES}^0}(t)\right\} + \frac{1}{2} \left\langle \left(W_{ES}^0\right)^2 \right\rangle \ . \tag{60}$$

We now assume that $f_{W_{ES}^0}(t)$ decays to zero "fast enough" in the sense that there exists some $\varepsilon > 0$ such that $\lim\limits_{t \to \infty} \left\{ f_{W_{ES}^0}(t)/t^{-(3+\varepsilon)} \right\} = 0$. This condition asserts that $\lim\limits_{t \to \infty} \left\{ \frac{1}{2} t^2 \bar{F}_{W_{ES}^0}(t) \right\} = \lim\limits_{t \to \infty} \left\{ \frac{1}{2} t^2 \int_t^\infty f_{W_{ES}^0}(z) dz \right\} = 0$ and that $\frac{1}{2} \int_0^\infty t^2 \cdot f_{W_{ES}^0}(t) dt = \frac{1}{2} \left\langle \left(W_{ES}^0 \right)^2 \right\rangle < \infty$. Substituting back into Eq. (59) we obtain

$$\langle M \rangle = \frac{1}{2} \frac{\left\langle \left(W_{ES}^0 \right)^2 \right\rangle}{\langle W_{ES}^0 \rangle} = \frac{1}{2} \langle W_{ES}^0 \rangle \left(CV_{W_{ES}^0}^2 + 1 \right) , \tag{61}$$

where $CV_{W_{ES}^0}^2 \equiv \frac{\sigma^2(W_{ES}^0)}{\langle W_{ES}^0 \rangle^2} = \frac{\left\langle \left(W_{ES}^0 \right)^2 \right\rangle - \langle W_{ES}^0 \rangle^2}{\langle W_{ES}^0 \rangle^2}$ is the normalized variance, a.k.a coefficient of variation, of W_{ES}^0.

Substituting Eqs. (57) and (61) back into Eq. (53) we rewrite the condition for the emergence of inhibitor-activator duality in terms of the mean, conditional mean, and fluctuations in the life time of the ES complex in the absence of inhibition

$$\frac{1}{2} \langle W_{ES}^0 \rangle \left(CV_{W_{ES}^0}^2 + 1 \right) >$$

$$\frac{k_{on}^{EI} K_m \langle T_{off}^{EI} \rangle}{k_{on}^{ESI}[S]} + \left(1 + \frac{K_m}{[S]} \right) \langle W_{ES}^0 | ES \to E + P \rangle + \left\langle T_{off}^{ESI} \right\rangle - \frac{1}{k_{on}[S]} . \tag{62}$$

Rearranging and recalling that $K_m k_{on} = \langle W_{ES}^0 \rangle^{-1}$ we get

$$\frac{\langle T_{mix} \rangle + \left\langle T_{off}^{ESI} \right\rangle}{\langle W_{ES}^0 \rangle} < \frac{1}{2} \left(CV_{W_{ES}^0}^2 - 1 \right) + \left(1 - \frac{\langle W_{ES}^0 | ES \to E + P \rangle}{\langle W_{ES}^0 \rangle} \right) \left(1 + \frac{K_m}{[S]} \right) , \tag{63}$$

where $\langle T_{mix} \rangle \equiv \frac{k_{on}^{EI} K_m}{k_{on}^{ESI}[S]} \left\langle T_{off}^{EI} \right\rangle$. Finally, in the case of uncompetitive inhibition $k_{on}^{EI} = 0$, $\langle T_{mix} \rangle = 0$, and one is left with

$$\frac{\left\langle T_{off}^{ESI} \right\rangle}{\langle W_{ES}^0 \rangle} < \frac{1}{2} \left(CV_{W_{ES}^0}^2 - 1 \right) + \left(1 - \frac{\langle W_{ES}^0 | ES \to E + P \rangle}{\langle W_{ES}^0 \rangle} \right) \left(1 + \frac{K_m}{[S]} \right) \tag{64}$$

which coincides with Eq. (4) in the main text.