

Supporting Information

On Kinetic Constraints that Catalysis Imposes on Elementary Processes

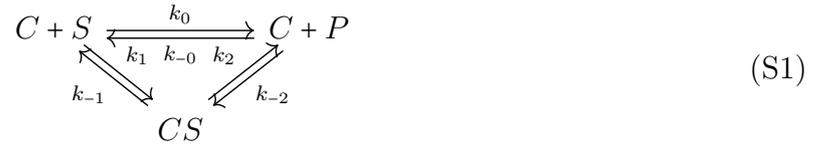
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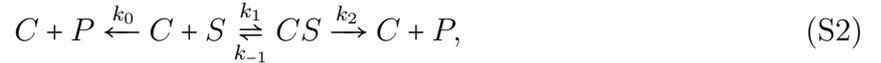
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I. MARKOVIAN CATALYTIC CYCLES

The simplest catalytic cycle is Markovian, and comprises only one intermediate state denoted as CS , which is accessible from either the unbound states $C + S$ or $C + P$ and can also transition back to those states. Graphically, the cycle is represented as



with k_{+n} denoting the forward rate of an elementary reaction and k_{-n} its reverse rate. Given that we consider the mean first-passage time $T_{C+S \rightarrow C+P}$ from the initial state $C + S$ to the absorbing state $C + P$, the transitions with rates k_{-0} and k_{-2} can be ignored and an equivalent representation is



where $C + P$ is repeated on both sides.

When in $C + S$, the mean time before any transition is $T_{C+S} = 1/(k_0 + k_1)$, the probability to transition to CS is $p_{C+S \rightarrow CS} = k_1/(k_0 + k_1)$ and the probability to transition directly to $C + P$ is $p_{C+S \rightarrow C+P} = 1 - p_{C+S \rightarrow CS} = k_0/(k_0 + k_1)$.¹ Similarly, when in CS , the mean time before any transition is $T_{CS} = 1/(k_2 + k_{-1})$, the probability to transition to $C + P$ is $p_{CS \rightarrow C+P} = k_2/(k_2 + k_{-1})$ and the probability to transition back to $C + S$ is $p_{CS \rightarrow C+S} = k_{-1}/(k_2 + k_{-1})$. In terms of these quantities, the mean first-passage time from $C + S$ to $C + P$, denoted $T_{C+S \rightarrow C+P}$, can be expressed as a function of the mean first-passage time from CS to $C + P$, denoted $T_{CS \rightarrow C+P}$ as^{2,3}:

$$T_{C+S \rightarrow C+P} = T_{C+S} + p_{C+S \rightarrow CS} T_{CS \rightarrow C+P} \tag{S3}$$

$$T_{CS \rightarrow C+P} = T_{CS} + p_{CS \rightarrow C+S} T_{C+S \rightarrow C+P}, \tag{S4}$$

i.e.,

$$T_{C+S \rightarrow C+P} = \frac{1}{k_0 + k_1} + \frac{k_1}{k_0 + k_1} T_{CS \rightarrow C+P} \quad (\text{S5})$$

$$T_{CS \rightarrow C+P} = \frac{1}{k_1 + k_2} + \frac{k_{-1}}{k_1 + k_2} T_{C+S \rightarrow C+P}, \quad (\text{S6})$$

Solving these equations lead to an explicit expression of $T_{C+S \rightarrow C+P}$ as a function of the elementary rates:

$$T_{C+S \rightarrow C+P} = \frac{k_1 + k_{-1} + k_2}{k_0 k_{-1} + k_0 k_2 + k_1 k_2}. \quad (\text{S7})$$

To highlight constraints, we can rewrite Eq. (S7) as follows:

$$T_{C+S \rightarrow C+P} = T_{S \rightarrow P} + \rho_{\text{cat}} (T_{\text{cat}} - T_{S \rightarrow P}), \quad (\text{S8})$$

with $T_{S \rightarrow P} = 1/k_0$, $T_{\text{cat}} = 1/k_2$ and

$$\rho_{\text{cat}} = \frac{1}{1 + (1 + k_{-1}/k_2)k_0/k_1}, \quad (\text{S9})$$

where T_{cat} denotes the mean time to form the product once the substrate is bound to the autocatalyst, while ρ_{cat} denotes the probability to reach $C + P$ from $C + S$ through the catalytic pathway ($CS \rightarrow C + P$). Indeed, let $p_1 = k_1/(k_0 + k_1)$ be the probability to transition from $C + S$ to CS and $p_2 = k_2/(k_{-1} + k_2)$ the probability to transition from CS to $C + P$. The probability to reach $C + P$ from $C + S$ through the $CS \rightarrow C + P$ is:

$$\rho_{\text{cat}} = p_1 \left(\sum_{n=0}^{\infty} (1 - p_2)^n p_1^n \right) p_2 = \frac{p_1 p_2}{1 - (1 - p_2) p_1} = \frac{1}{1 + (1 + k_{-1}/k_2)k_0/k_1}. \quad (\text{S10})$$

where n is the number of times the back transition $CS \rightarrow C + S$ occurs.

II. GENERAL CRITERION FOR CATALYSIS

We present here the derivation of Eq. (S8) for the general case where the spontaneous reaction $S \rightarrow P$ occurs at a given rate. A single S and a single C are considered. We assume that a state $C \cdot S$ can be defined as a set of configurations where C and S are not interacting and where the probability and the nature of their future interactions are equivalent. This state defines a boundary between interacting and non-interacting systems. The initial configuration of the system denoted $(C + S)_0$, is assumed to be a non-interacting configuration and the final configuration, as well the final configuration $C + P$. From $(C + S)_0$, the system may either reach $C \cdot S$ by diffusion or reach $C + P$ through the spontaneous reaction $S \rightarrow P$. Once in $C \cdot S$, C and S may either move apart to $C \cdot S^+$ or move closer to $C \cdot S^-$, where $C \cdot S^+$ and $C \cdot S^-$ represent configurations infinitesimally close to $C \cdot S$. From $C \cdot S^+$, the system may either come back to $C \cdot S$ or reach the absorbing state $C + P$ through a spontaneous reaction. From $C \cdot S^-$, on the other hand, it may either come back to $C \cdot S$ or reach the absorbing state $C + P$ through catalysis. We will compare this situation to a situation where $C \cdot S^+$ is inaccessible, which may for instance be implemented in numerical simulations by confining the system in a box with reflecting boundaries defined by $C \cdot S$: the

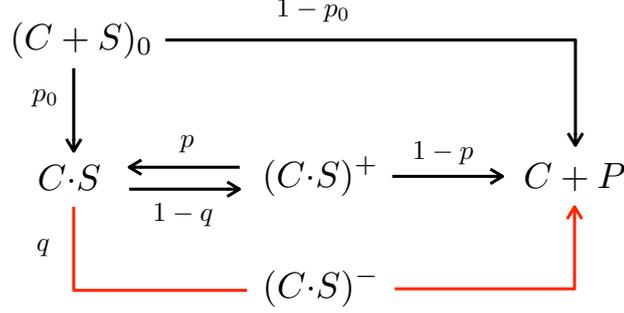


FIG. S1: States in the derivation of the necessary and sufficient condition on catalysis. We consider 3 intermediate states in addition to the initial state $(C+S)_0$ and the final absorbing state $C+P$. The probabilities to transition between these states are indicated next to the arrows. The times to transition from $(C+S)_0$ to $C\cdot S$ given $p_0 = 1$, from $C\cdot S^+$ to $C\cdot S$ given $p = 1$ and from $C\cdot S$ to $C+P$ given $q = 1$ may follow arbitrary distributions, but the times to transition from S to P in absence of C are assumed to be exponentially distributed. The time to transition from $C\cdot S$ to $(C\cdot S)^+$ given $q = 0$ is assumed to be negligible. The mean time $T_{(C+S)_0 \rightarrow C+P}$ to reach $C+P$ from $(C+S)_0$ is expressed in terms of the overall probability $\rho_{\text{cat}} = p_0 q / (1 + (1-q)p)$ to take the catalytic (red) route and of the mean time T_{cat} to transition from $C\cdot S$ to $C+P$ given $q = 1$ as $T_{(C+S)_0 \rightarrow C+P} = T_{S \rightarrow P} + \rho_{\text{cat}}(T_{\text{cat}} - T_{S \rightarrow P})$ where $T_{S \rightarrow P}$ is the mean time to reach P from S in absence of C .

mean time to reach $C+P$ from $C\cdot S$ in this case is what we define as T_{cat} and also denote as $T_{C\cdot S \rightarrow C+P \setminus (C+S)}$, where $\setminus (C+S)$ indicates that the configurations $C+S$ are excluded.

To derive Eq. (S8) we make the following hypotheses: $S \rightarrow P$ occurs at a rate k_0 , $(C+S)_0 \rightarrow C\cdot S$ takes a mean time $T'_{(C+S)_0 \rightarrow C\cdot S}$ and $C\cdot S^+ \rightarrow C\cdot S$ a mean time $T'_{C\cdot S^+ \rightarrow C\cdot S}$, both in absence of spontaneous reaction. Eq. (S8) then results from combining the following three equations which are derived below.

First, we have

$$T_{(C+S)_0 \rightarrow C+P} = p_0 T_{C\cdot S \rightarrow C+P} + (1-p_0) T_{S \rightarrow P} \quad (\text{S11})$$

where p_0 is the probability to reach $C\cdot S$ from $(C+S)_0$ before any spontaneous reaction (Fig. 1).

Second, we have

$$T_{C\cdot S \rightarrow C+P} = q T_{C\cdot S \rightarrow C+P \setminus (C+S)} + (1-q) T_{C\cdot S^+ \rightarrow C+P} \quad (\text{S12})$$

where q is the probability to reach $C+P$ from $C\cdot S$ without ever revisiting $C\cdot S^+$ (Fig. 1). Here $T_{C\cdot S \rightarrow C+P \setminus (C+S)}$ could also be written $T_{C\cdot S \rightarrow C+P \setminus (C\cdot S^+)}$ and the assumption is that $T_{C\cdot S \rightarrow C\cdot S^+ \setminus (C\cdot S)^-} = 0$.

Third, we have

$$T_{C\cdot S^+ \rightarrow C+P} = p T_{C\cdot S \rightarrow C+P} + (1-p) T_{S \rightarrow P} \quad (\text{S13})$$

where p is the probability to come back to $C\cdot S$ after an excursion in $C+S$ before any spontaneous reaction (Fig. 1).

Combining these three equations leads to

$$T_{(C+S)_0 \rightarrow C+P} = \rho T_{C\cdot S \rightarrow C+P \setminus (C+S)} + (1-\rho) T_{S \rightarrow P} \quad (\text{S14})$$

which is equivalent to Eq. (S8), with

$$\rho = \frac{p_0 q}{1 - (1 - q)p}. \quad (\text{S15})$$

To interpret ρ , note that it may also be written

$$\rho = \sum_{n=0}^{\infty} p_0 (1 - q)^n p^n q \quad (\text{S16})$$

where the sum is over the probabilities that $(C + S)_0$ reaches $C \cdot S$ (factor p_0), “unbind” n times to $C + S$ (factor $(1 - q)^n$) and “rebinds” as many times to $C \cdot S$ (factor p^n) before following the catalytic route towards $C + P$ (factor q): ρ therefore represents the overall probability to reach $C + P$ through catalysis when starting from $(C + S)_0$.

A. Derivation of Eq. (S11)

Consider that starting from $(C + S)_0$ the system takes a mean time $T_{(C+S)_0}$ before it either reaches $C \cdot S$, with probability p_0 , or $C + P$, with probability $1 - p_0$ so that

$$T_{(C+S)_0 \rightarrow C+P} = T_{(C+S)_0} + p_0 T_{C \cdot S \rightarrow C+P}. \quad (\text{S17})$$

Eq. (S11) is then obtained by noting that $T_{(C+S)_0} = (1 - p_0)T_{S \rightarrow P}$. To show this later relation, the essential ingredient is that the spontaneous reaction occurs within a time t that is exponentially distributed with a rate k_0 such that $T_{S \rightarrow P} = 1/k_0$. The time τ for $(C + S)_0$ to diffuse towards $C \cdot S$ in absence of any possible spontaneous reaction may, on the other hand, follow an arbitrary distribution $\chi(\tau)$. Under these assumptions, we have

$$p_0 = \int_0^{\infty} d\tau \chi(\tau) \int_{\tau}^{\infty} dt k e^{-kt} = \int_0^{\infty} d\tau \chi(\tau) e^{-k\tau} = \langle e^{-k\tau} \rangle \quad (\text{S18})$$

where $\langle \cdot \rangle$ denotes an average over τ based on $\chi(\tau)$, and

$$T_{(C+S)_0} = \int_0^{\infty} d\tau \chi(\tau) \int_0^{\infty} dt k_0 e^{-k_0 t} [t 1(t < \tau) + \tau 1(\tau < t)] \quad (\text{S19})$$

$$= \langle \int_0^{\tau} dt t k_0 e^{-k_0 t} + \tau \int_{\tau}^{\infty} dt k_0 e^{-k_0 t} \rangle = \frac{1}{k_0} (1 - \langle e^{-k_0 \tau} \rangle) \quad (\text{S20})$$

from which it follows that $T_{(C+S)_0} = (1 - p_0)T_{S \rightarrow P}$.

Note that if τ is exponentially distributed with mean $1/k_1$ we have simply $p_0 = k_1/(k_0 + k_1)$ and $T_{(C+S)_0} = 1/(k_0 + k_1)$ which leads directly to $T_{(C+S)_0} = (1 - p_0)T_{S \rightarrow P}$.

B. Derivation of Eq. (S12)

Let α be the probability that when in $C \cdot S$ the system goes towards $C \cdot S^-$ rather than towards $C \cdot S^+$ (in no time) and let β be the probability that when in $C \cdot S^-$ the system will come back at least once to $C \cdot S$. By definition,

$$T_{C \cdot S \rightarrow C+P} = \alpha T_{C \cdot S^- \rightarrow C+P} + (1 - \alpha) T_{C \cdot S^+ \rightarrow C+P} \quad (\text{S21})$$

$$T_{C \cdot S^- \rightarrow C+P} = \beta (T_{C \cdot S^- \rightarrow C \cdot S} + T_{C \cdot S \rightarrow C+P}) + (1 - \beta) T_{C \cdot S^- \rightarrow C+P \setminus C \cdot S} \quad (\text{S22})$$

where $T_{C \cdot S^- \rightarrow C+P \setminus C \cdot S}$ denotes the time to reach $C+P$ from $C \cdot S^-$ without ever visiting $C \cdot S$. From these equations it follows that

$$T_{C \cdot S \rightarrow C+P} = \frac{1}{1 - \alpha\beta} \left(\alpha(\beta T_{C \cdot S^- \rightarrow C \cdot S} + (1 - \beta)T_{C \cdot S^- \rightarrow C+P \setminus C \cdot S}) + (1 - \alpha)T_{C \cdot S^+ \rightarrow C+P} \right). \quad (\text{S23})$$

The point is that $T_{\text{cat}} = T_{C \cdot S \rightarrow C+P \setminus C \cdot S}$ can be written in the same way but with $\alpha = 1$ since it correspond to a case where accessing $C \cdot S^+$ is excluded,

$$T_{\text{cat}} = \frac{1}{1 - \beta} \left[\beta T_{C \cdot S^- \rightarrow C \cdot S} + (1 - \beta)T_{(C \cdot S^- \rightarrow C+P) \setminus C \cdot S} \right] \quad (\text{S24})$$

This leads to

$$T_{C \cdot S \rightarrow C+P} = \frac{1}{1 - \alpha\beta} \left[\alpha(1 - \beta)T_{\text{cat}} + (1 - \alpha)T_{C \cdot S^+ \rightarrow C+P} \right] \quad (\text{S25})$$

which is equivalent to Eq. (S13) with

$$q = \frac{\alpha(1 - \beta)}{1 - \alpha\beta} = \alpha \left(\sum_{n=0}^{\infty} \beta^n \alpha^n \right) (1 - \beta) \quad (\text{S26})$$

which can be interpreted as the probability to go from $C \cdot S$ to $C \cdot S^-$ (factor α), come back n times to $C \cdot S$ (factor β^n) and in each case immediately diffuse to $C \cdot S^-$ (factor α^n) an arbitrary number n of times (sum over n) before eventually reaching $C+P$ (factor $1 - \beta$), i.e., q is the probability to reach $C+P$ from $C \cdot S$ without ever visiting $C \cdot S^+$.

C. Derivation of Eq. (S13)

The derivation of Eq. (S13) is essentially equivalent to the derivation of Eq. (S11) with a starting point $C \cdot S^+$ instead of $(C+S)_0$, with p the probability to reach $C \cdot S$ replacing p_0 and with $\chi(\tau)$ now representing the distribution of times to reach $C \cdot S$ from $C \cdot S^+$ in absence of any possible spontaneous reaction.

III. CATALYSIS FOR DIMER DISSOCIATION

We consider here the catalytic cycle represented in Fig. 1. To derive necessary and sufficient conditions, we start by applying Eq. (S8) twice,

$$T_{C+S \rightarrow C+P} = (1 - \rho_0)T_{S \rightarrow P} + \rho_0 T_{C \cdot S \rightarrow C+P \setminus C+S} \quad (\text{S27})$$

$$T_{C \cdot S \rightarrow C \cdot P \setminus C+S} = (1 - \rho_1)T_{S \rightarrow P} + \rho_1 T_{C \cdot S \rightarrow C \cdot P \setminus C \cdot S} \quad (\text{S28})$$

and then relate $T_{C \cdot S \rightarrow C+P \setminus C+S}$ to $T_{C \cdot S \rightarrow C \cdot P \setminus C+S}$ by introducing $\gamma = \mathbb{P}(C \cdot P \rightarrow C \cdot S \setminus C+S)$:

$$T_{C \cdot S \rightarrow C+P \setminus C+S} = T_{C \cdot S \rightarrow C \cdot P \setminus C+S} + T_{C \cdot P \rightarrow C+P \setminus C+S} \quad (\text{S29})$$

$$T_{C \cdot P \rightarrow C+P \setminus C+S} = \gamma(T_{C \cdot P \rightarrow C \cdot S \setminus C+S} + T_{C \cdot S \rightarrow C+P \setminus C+S}) + (1 - \gamma)T_{C \cdot P \rightarrow C+P \setminus C \cdot S} \quad (\text{S30})$$

i.e.,

$$T_{C \cdot S \rightarrow C+P \setminus C+S} = \frac{1}{(1 - \gamma)} \left(T_{C \cdot S \rightarrow C \cdot P \setminus C+S} + \gamma T_{C \cdot P \rightarrow C \cdot S \setminus C+S} + (1 - \gamma)T_{C \cdot P \rightarrow C+P \setminus C \cdot S} \right) \quad (\text{S31})$$

All together,

$$T_{C+S \rightarrow C+P} = \left(1 - \rho_0 \frac{\rho_1 - \gamma}{1 - \gamma}\right) T_{S \rightarrow P} + \frac{\rho_0}{1 - \gamma} \left(\rho_1 T_{C:S \rightarrow C \cdot P \setminus C \cdot S} + \gamma T_{C \cdot P \rightarrow C \cdot S \setminus C+S} + (1 - \gamma) T_{C \cdot P \rightarrow C+P \setminus C \cdot S}\right) \quad (\text{S32})$$

which can also be written in the form $T_{C+S \rightarrow C+P} = (1 - \rho_{\text{cat}}) T_{S \rightarrow P} + \rho_{\text{cat}} T_{\text{cat}}$ with

$$\rho_{\text{cat}} = \rho_0 \left(1 - \frac{1 - \rho_1}{1 - \gamma}\right) = \rho_0 \left(1 - (1 - \rho_1) \sum_{n=0}^{\infty} \gamma^n\right) \quad (\text{S33})$$

that can be interpreted as the probability for the reaction not to occur spontaneously, neither through $C + S \rightarrow C + P$ nor through $C \cdot S \rightarrow C \cdot P$.

When $\gamma = 0$, for instance because the reaction is irreversible, this simplifies to $\rho_{\text{cat}} = \rho_0 \rho_1$ and $T_{\text{cat}} = T_{C:S \rightarrow C \cdot P \setminus C \cdot S} + \rho_1^{-1} T_{C \cdot P \rightarrow C+P \setminus C \cdot S}$ and a necessary condition for catalysis is therefore $T_{C:S \rightarrow C \cdot P \setminus C \cdot S} < T_{S \rightarrow P}$, which implies $T_{C:S \rightarrow C \cdot P \setminus C \cdot S} < T_{S \rightarrow P}$ and $T_{C \cdot P \rightarrow C \cdot S \setminus C+S} < T_{S \rightarrow P}$. On the other hand, there is no corresponding constraint on $T_{C \cdot S \rightarrow C:S \setminus C+S}$. Instead, a necessary condition on the transition $C \cdot S \rightarrow C:S$ takes the form $\rho_1 > T_{C \cdot P \rightarrow C \cdot P \setminus C \cdot S} / T_{S \rightarrow P}$.

IV. EXAMPLE OF A CATALYTIC CYCLE WITH AN OUT-OF-CYCLE INTERMEDIATE

Here we derive the distribution of the first passage times $\mathbb{P}[t_{\text{cat}}]$ for the catalytic cycle with an out-of-cycle intermediate presented in Fig 3, showing that when the distribution t_{cat} is not exponentially distributed, a molecule C that is not a catalyst in a single copy with a single substrate may be a catalyst in a condition with $n_c \geq 1$ catalysts and $n_s \geq 1$ substrates.

The catalytic scheme and notations for the states and rates are presented in Fig 3A. $\mathbb{P}[t_{\text{cat}} = t] = \partial P_{C+P}(t) / \partial t$ where $P_{C+P}(t)$ is obtained by considering the probabilities to be in each state at a function of time when starting at $t = 0$ from $C_1 S$ and when ignoring state $C + S$:

$$\begin{cases} \partial_t P_{CS}(t) = -(k_2^+ + k_3^+) P_{CS}(t) + k_3^- P_{C_1 S}(t) \\ \partial_t P_{C_1 S}(t) = k_3^+ P_{CS}(t) - k_3^- P_{C_1 S}(t) \\ \partial_t P_{C+P}(t) = k_2^+ P_{CS}(t) \end{cases} \quad (\text{S34})$$

with $P_{CS}(0) = 1$, $P_{C_1 S}(0) = 0$, and $P_{C+P}(0) = 0$.

The first two equations are decoupled from the third and are turned into a system of linear equation by considering the Laplace transforms $\hat{P}(s) = \int_0^{\infty} P(t) e^{-st}$ of the probability densities $P(t)$ ⁴:

$$\begin{cases} s \hat{P}_{CS}(s) - 1 = -(k_2^+ + k_3^+) \hat{P}_{CS}(s) + k_3^- \hat{P}_{C_1 S}(s) \\ s \hat{P}_{C_1 S}(s) = k_3^+ \hat{P}_{CS}(s) - k_3^- \hat{P}_{C_1 S}(s) \end{cases} \quad (\text{S35})$$

Solving these equations and applying the inverse Laplace transform, we obtain $P_{C_1 S}(t)$ and finally $\mathbb{P}[t_{\text{cat}} = t] = k_2^+ P_{CS}(t)$:

$$\mathbb{P}[t_{\text{cat}} = t] = \frac{A k_2^+}{2B} \left((-k_2^+ - k_3^+ + k_3^- + A) e^{-(k_2^+ + k_3^+ + k_3^- - A)t/2} - (-k_2^+ - k_3^+ + k_3^- - A) e^{-(k_2^+ + k_3^+ + k_3^- + A)t/2} \right) \quad (\text{S36})$$

where $A = \sqrt{-4k_2^+ k_3^- + (k_2^+ + k_3^+ + k_3^-)^2}$ and $B = (k_2^+)^2 + (k_3^+)^2 + (k_3^-)^2 + 2k_2^+ k_3^+ - 2k_2^+ k_3^- + 2k_3^+ k_3^-$. This is the probability density represented in Fig 3B.

In the limit $k_3^- \ll k_2^+, k_3^+$, it simplifies to

$$\mathbb{P}[t_{\text{cat}} = t] \simeq k_2^+ \left(e^{-(k_2^+ + k_3^+)t} + \frac{k_3^- k_3^+}{(k_2^+ + k_3^+)^2} e^{-k_2^+ k_3^- t / (k_2^+ + k_3^+)} \right) \quad (\text{S37})$$

and if further assuming $k_3^+ \ll k_2^+$,

$$\mathbb{P}[t_{\text{cat}} = t] \simeq k_2^+ \left(e^{-k_2^+ t} + \frac{k_3^- k_3^+}{(k_2^+)^2} e^{-k_3^- t} \right) \quad (\text{S38})$$

This shows that $\mathbb{P}[t_{\text{cat}}]$ has two time scales: $1/k_2^+$ at short times and $1/k_3^-$ at long times.

V. CONDITIONS FOR CATALYSIS IN PRESENCE OF MULTIPLE CATALYSTS OR MULTIPLE SUBSTRATES

We consider here the particular case where either the substrate or the catalyst is in a single copy and show that for three elementary reactions catalysis occurs in presence of a single substrate and multiple catalysts or in presence of a single catalyst and multiple substrates *only if* it occurs with a single substrate and a single catalyst. The three reactions are a unimolecular reaction, a reaction with a bimolecular substrate, and another with bimolecular products. We assume a decomposition of the catalytic cycles as in Fig. 2, and further assume them to be Markovian. The rate ρ_i for each transition i is the product of the constant reaction rate k_i and the numbers of reactant(s), e.g., $\rho_1^+ = k_1^+ n[C][S]$. Importantly, the conclusion obtained for these reactions does *not* extend to any spontaneous reaction, as the counter-example of Fig. 3 shows.

The conclusion can be drawn from the inspection of Fig. 2 when noting that the introduction of multiple catalysts (second row) or multiple substrates (third row) effectively modifies the catalytic cycle with a single substrate and a single catalyst (first row) in three possible ways. By modifying some of the rates (represented by the black arrows of large size), by adding additional states, or by effectively increasing the rate of the spontaneous reaction (when increasing the number of S). Crucially, however, the added states are only increasing the time to complete the catalytic route, and the modified rate in the main cycle are confined to ρ_0^+ to ρ_1^+ but ρ_0^+ only increases with the number of substrates, which effectively makes catalysis harder, while ρ_1^+ is involved in the efficiency of catalysis but not in the criterion for the presence of catalysis (see section III A 3). These observations imply that in all three cases catalysis occurs in presence of a single substrate and multiple catalysts or in presence of a single catalyst and multiple substrates only if it occurs with a single substrate and a single catalyst.

To demonstrate it more formally in one example, consider for instance the case of the reaction with a bimolecular substrate when considering a varying number n of catalysts C and a single substrate S (central box in Fig. 2). In this case, ρ_1^+ is effectively multiplied by a factor n and an extra (futile) state has to be considered where two different catalysts are bound by one substrate each. The transition to this out-of-cycle intermediate $2CS + (n-2)C$ from $CS + S + (n-1)S$ occurs at rate $\rho_{01}^+ = \rho_1^+(n-1)$ and release from this state at a rate $\rho_{01}^- = 2\rho_1^-$. The condition for catalysis, $T_{n_c C + S \rightarrow n_c C + P} < T_{S \rightarrow P}$, can be derived by solving the system of equations associated with the reaction scheme (as in Section ??). It leads to

$$\frac{1}{\rho_2^+} + \frac{1}{\rho_3^+} + \frac{\rho_2^-}{\rho_2^+ \rho_3^+} + \frac{\rho_{01}^+ (\rho_2^- + \rho_3^+)}{\rho_{01}^- (\rho_2^+ \rho_3^+)} < \frac{1}{\rho_0^+} \quad (\text{S39})$$

	Unimolecular	Bimolecular Substrate	Bimolecular Product
(1S, 1C)	$C + S \xrightleftharpoons[\rho_0^+]{\rho_1^+} C + P$ $\rho_1^- \uparrow \downarrow \rho_1^+$ $CS \xrightleftharpoons[\rho_2^+]{\rho_2^-} CP$	$C + 2S \xrightleftharpoons[\rho_0^+]{\rho_1^+} C + P$ $\rho_1^- \uparrow \downarrow \rho_1^+$ $CS + S \xrightleftharpoons[\rho_2^+]{\rho_2^-} CS_2$	$C + S \xrightleftharpoons[\rho_0^+]{\rho_1^+} C + 2P$ $\rho_1^- \uparrow \downarrow \rho_1^+$ $CS \xrightleftharpoons[\rho_2^+]{\rho_2^-} CP + P$
(1S, nC)	$nC + S \xrightleftharpoons{\rho_1^+} nC + P$ $\uparrow \downarrow$ $CS + (n-1)C \xrightleftharpoons{\rho_2^+} CP + (n-1)C$	$nC + 2S \xrightleftharpoons{\rho_1^+} C + P$ $\uparrow \downarrow$ $CS + S + (n-1)C \xrightleftharpoons{\rho_2^+} CS_2 + (n-1)C$ $\uparrow \downarrow$ $2CS + (n-2)C$	$nC + S \xrightleftharpoons{\rho_1^+} C + 2P$ $\uparrow \downarrow$ $CS + (n-1)C \xrightleftharpoons{\rho_2^+} CP + (n-1)C + P$ $\uparrow \downarrow$ $2CP + (n-2)C$
(nS, 1C)	$C + nS \xrightleftharpoons{\rho_1^+} C + (n-1)S + P$ $\uparrow \downarrow$ $CS + (n-1)S \xrightleftharpoons{\rho_2^+} CP + (n-1)S$ $\uparrow \downarrow$ $CS + (n-2)S + P \quad CP + (n-2)S + P$	$C + nS \xrightleftharpoons{\rho_1^+} C + (n-2)S + P$ $\uparrow \downarrow$ $CS + (n-1)S \xrightleftharpoons{\rho_2^+} CP + (n-2)S$ $\uparrow \downarrow$ $CS + (n-3)S + P \quad CS + (n-4)S + P$	$C + nS \xrightleftharpoons{\rho_1^+} C + (n-1)S + 2P$ $\uparrow \downarrow$ $CS + (n-1)S \xrightleftharpoons{\rho_2^+} CP + (n-1)S + P$ $\uparrow \downarrow$ $CS + (n-2)S + 2P \quad CP + (n-2)S + 3P$

FIG. S2: Catalytic cycles for three particular reactions: a unimolecular reaction, a reaction with a bimolecular substrate, and another with bimolecular products. In the first row, we initiate the systems with one substrate and one catalyst; in the second row, with one substrate and n catalysts; in the third row, with one catalyst and n substrates. The rate ρ_i for each transition i is the product of the constant reaction rate k_i and the numbers of reactant(s), e.g., $\rho_1^+ = k_1^+ n[C][S]$.

We thus verify that ρ_1^+ is not involved and that the presence of an out-of-cycle intermediate makes the criterion for catalysis more stringent compared to the case with a single catalyst for which this criterion is simply

$$\frac{1}{\rho_2^+} + \frac{1}{\rho_3^+} + \frac{\rho_2^-}{\rho_2^+ \rho_3^+} < \frac{1}{\rho_0^+} \quad (\text{S40})$$

where the rates ρ_2^+ , ρ_2^- and ρ_3^+ are unchanged. Similar derivations can be done for the other cases.

We have considered here bimolecular reactions where the substrates or products are indistinguishable. If they are distinct and if their binding occur sequentially, i.e., $C + S_1 + S_2 \leftrightarrow CS_1 \leftrightarrow CS_1 S_2 \leftrightarrow C + P$ or $C + S \leftrightarrow CS \leftrightarrow CP_1 + P_2 \leftrightarrow C + P_1 + P_2$, then no out-of-cycle intermediates are present.

In Fig. 4, we computed the mean-first passage time for each reaction scheme in Fig. 2 and used the following reaction rates: for the unimolecular reaction with no out-of-cycle intermediate, $k_0^+ = 0.15$, $k_1^+ = 2$, $k_1^- = 1$, $k_2^+ = 1$, $k_2^- = 1$, and $k_3^+ = 1$; for the reaction with a bimolecular product, $k_0^+ = 0.15$, $k_1^+ = 0.5$, $k_1^- = 1$, $k_2^+ = 1$, $k_2^- = 1$, $k_3^+ = 1$, and $k_3^- = 0.3$; for the reaction with a bimolecular substrate, $k_0^+ = 0.08$, $k_1^+ = 0.1$, $k_1^- = 1$, $k_2^+ = 1$, $k_2^- = 1$, and $k_3^+ = 1$. For the unimolecular reaction with an out-of-cycle intermediate, we set $k_0^+ = 0.15$, $k_2^+ = 1$, $k_3^+ = 0.001$, $k_3^- = 0.0001$.

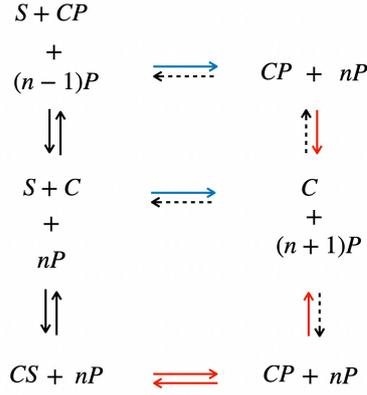


FIG. S3: Catalytic cycle with multiple products for a unimolecular reaction $S \rightarrow P$ initiated with a single substrate, a single catalyst, and n products. The difference with the case of no product ($n = 0$) is the upper path that includes the spontaneous reaction together with the binding and unbinding of a product to the catalyst.

VI. CATALYSIS IN PRESENCE OF MULTIPLE PRODUCTS

To illustrate how catalysis in presence of multiple products requires catalysis to take place in presence of no product, consider for instance the unimolecular reactions $S \rightarrow P$ represented in Fig. 3, with initially one substrate S , one catalyst C and n products P . The final state of interest is $C + (n+1)P$. This state is reached either through the spontaneous reaction, the catalyzed reaction (path at the bottom) or through the upper path, which corresponds to the spontaneous reaction plus the binding/unbinding of the catalyst to a product.

The conclusion follows from a simple observation: the presence of products only opens an addition path (the upper path of Fig. 3), in addition to the spontaneous (middle) and catalytic (lower) paths that are present in absence of the product. As this additional path includes the spontaneous reaction together with the binding and unbinding of the catalyst to a product, it cannot be faster than the spontaneous reaction. As a consequence, catalysis must rely on the lower path, which is the same as in absence of products. $T_{\text{cat}} < T_{S \rightarrow P}$ is therefore necessary for catalysis irrespectively of the number n of products.

VII. CATALYSIS BEYOND THE FIRST PRODUCT

We have represented in Fig. 5A the formation of 3 products from 3 substrate molecules with 3 catalysts, when products are removed upon formation, and in the context of an irreversible spontaneous reaction. In this scheme, we see that the dynamics essentially boils down to moving from one column to the one on its right. We have shown that this can be faster than the corresponding spontaneous reactions (reactions on the top of the columns) only if there is at least one state on a left column that departs to a state on the right faster than the spontaneous reaction (Eq. (13)). A sufficient condition for catalysing n_p reaction is that each of these moves is faster than their corresponding spontaneous reaction. A necessary condition is that the mean time for n_p spontaneous reactions to proceed is longer than a combination of moves:

$$\begin{aligned} & \mathbb{E}[\min(t_{S \rightarrow P}^{(1)}, \dots, t_{S \rightarrow P}^{(n_s-r)}, t_{\text{cat}}^{(1)}, \dots, t_{\text{cat}}^{(r)})] + \dots + \mathbb{E}[\min(t_{S \rightarrow P}^{(1)}, \dots, t_{S \rightarrow P}^{(n_s-r-n_p)}, t_{\text{cat}}^{(1)}, \dots, t_{\text{cat}}^{(r-n_p)})] \\ & < \mathbb{E}[\min(t_{S \rightarrow P}^{(1)}, \dots, t_{S \rightarrow P}^{(n_s)})] + \dots + \mathbb{E}[\min(t_{S \rightarrow P}^{(1)}, \dots, t_{S \rightarrow P}^{(n_s-n_p)})] \end{aligned}$$

However, we note that even in the simplest case where products are systematically removed whenever produced, the total time $T_{n_s S+n_c C \rightarrow (n_s-n_p)S+n_c C+n_p P}$ cannot be computed as a sum $\sum_{r=0}^{n_p} T_{(n_s-r)S+n_c C \rightarrow (n_s-n_p)S+n_c C+P}$.

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