Slow deactivation of RuBisCO elucidated by mathematical models

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S1 Model equations for the simple model

The kinetic behavior of RuBisCO is described by a system of ordinary differential equations. These equations are derived from mass balance considerations.
For the simple model represented in Fig. 1 of the main text, they read

$$d[ER]/dt = v_{ER} - v_{ERC} - v_{ERO} - v_{EI1} \tag{1}$$
$$d[ERC]/dt = v_{ERC} - v_{\mathrm{cat}} \tag{2}$$
$$d[ERO]/dt = v_{ERO} - v_{\mathrm{oxy}} - v_{EI2} \tag{3}$$
$$d[EI1]/dt = v_{EI1} + v_X \tag{4}$$
$$d[EI2]/dt = v_{EI2} + v_P. \tag{5}$$

The reaction rates are based on mass-action kinetics. They read

$$v_{ER} = k^+_{ER}[E][RuBP] - k^-_{ER}[ER] \tag{6}$$
$$v_{ERC} = k^+_{ERC}[ER][CO_2] - k^-_{ERC}[ERC] \tag{7}$$
$$v_{ERO} = k^+_{ERO}[ER][O_2] - k^-_{ERO}[ERO] \tag{8}$$
$$v_{cat} = k_{cat}[ERC] \tag{9}$$
$$v_{oxy} = k_{oxy}[ERO] \tag{10}$$
$$v_{EI1} = k^+_{EI1}[ER] - k^-_{EI1}[EI1] \tag{11}$$
$$v_{EI2} = k^+_{EI2}[ERO] - k^-_{EI2}[EI2] \tag{12}$$
$$v_X = k^+_X[E][XuBP] - k^-_X[EI1] \tag{13}$$
$$v_P = k^+_P[E][PDBP] - k^-_P[EI2] \tag{14}$$

In principle, all reactions are assumed to be reversible and thus depend on two kinetic parameters (denoted by $^+$ and $^-$ for the forward and backbard rate constant). The last catalytic steps of the carboxylation or oxygenation are assumed to be irreversible. While it should be in principle possible to reverse these steps by applying very large product concentrations, such a reverse activity has not been reported. Moreover, under *in-vivo* as well as *in-vitro* conditions, the products are rapidly processed by other enzymes, leading to a de-facto irreversibility of the final catalytic steps.
The concentrations of the substrates $RuBP$, $XuBP$, CO_2, O_2 are considered to be constants. This is realistic since in most experimental conditions substrates are applied in excess and side product formation proceeds extremely slow so that the change in concentration can be neglected on the time scale on which the experiments are performed.
The total amount of enzyme is also fixed. This results in a conserved quantity involving all molecular species containing the enzyme,

$$[E] + [ER] + [ERC] + [ERO] + [EI1] + [EI2] = E^{\mathrm{tot}}. \tag{15}$$

As a consequence, there are only five independent system variables. We decided to eliminate the concentration of the free enzyme, $[E]$, and expressed it through the other five system variables according to Eq. 15.

S2 Quasi steady state approximation

The variables $[ER]$, $[ERC]$ and $[ERO]$ are assumed to change fast when compared to the dynamics of the enzyme-inhibitor complexes $[EI_1]$ and $[EI_2]$. The quasi steady state approximation therefore implies

$$d[ER]/dt = 0, \quad d[ERC]/dt = 0, \quad d[ERO]/dt = 0. \tag{16}$$

Using equations 1–3, 6–12 and 15, this assumption results in the quasi steady state concentrations

$$[ER] = [ER]_i - a[EI_1] - b[EI_2] \tag{17}$$
$$[ERC] = \frac{k^+_{ERC}}{k^-_{ERC} + k_{cat}}[CO_2][ER] = \gamma[CO_2][ER] \tag{18}$$
$$[ERO] = \frac{k^+_{ERO}[O_2][ER] + k^-_{EI2}[EI_2]}{k^-_{ERO} + k^+_{EI2} + k_{oxy}} = \omega[O_2][ER] + \kappa[EI_2], \tag{19}$$

where the following abbreviations have been introduced

$$\gamma = \frac{k^+_{ERC}}{k^-_{ERC} + k_{cat}}, \tag{20}$$

$$\omega = \frac{k^+_{ERO}}{k^-_{ERO} + k^+_{EI2} + k_{oxy}}, \tag{21}$$

$$\kappa = \frac{k^-_{EI2}}{k^-_{ERO} + k^+_{EI2} + k_{oxy}}, \tag{22}$$

$$[ER]_i = \frac{k^+_{ER}[RuBP]E^{\mathrm{tot}}}{d}, \tag{23}$$

$$a = \frac{k^+_{ER}[RuBP] - k^-_{EI1}}{d}, \tag{24}$$

$$b = \frac{k^+_{ER}[RuBP](1+\kappa) - k^-_{ERO}\kappa}{d}, \tag{25}$$

$$\text{with} \quad d = k^+_{ER}[RuBP](1+\gamma[CO_2]+\omega[O_2]) + k^-_{ER} + k_{cat}\gamma[CO_2] + (k_{oxy}+k^+_{EI2})\omega[O_2] + k^+_{EI1}. \tag{26}$$

The subscript i in Eq. 23 denotes the initial concentration after rapid relaxation to the quasi steady state but before inhibitor formation. It is in the following also used for the concentrations of other complexes.

S2.1 Fast reaction dynamics

Expression 23 describes the concentration of the complex $[ER]$ after the rapid relaxation to the quasi steady state but before any significant amounts of inhibitors have been produced ($[EI_1] = [EI_2] = 0$). The initial rate of catalysis therefore reads

$$v_i = k_{cat}[ERC]_i = k_{cat}\gamma[CO_2][ER]_i = \frac{\gamma k_{cat}\frac{k^+_{ER}}{k^-_{ER}+k^+_{EI1}}E^{\mathrm{tot}}[RuBP][CO_2]}{1 + \frac{[RuBP]}{L_R} + \frac{[CO_2]}{L_C} + \frac{[O_2]}{L_O} + \frac{[RuBP][CO_2]}{L_{RC}} + \frac{[RuBP][O_2]}{L_{RO}}} \tag{27}$$

$$\text{with} \quad L_R = \frac{k^-_{ER} + k^+_{EI1}}{k^+_{ER}}, \tag{28}$$

$$L_C = \frac{k^-_{ER} + k^+_{EI1}}{\gamma k_{cat}}, \tag{29}$$

$$L_O = \frac{k^-_{ER} + k^+_{EI1}}{(k_{oxy} + k^+_{EI2})\omega}, \tag{30}$$

$$L_{RC} = \frac{L_R}{\gamma} = \frac{k^-_{ER} + k^+_{EI1}}{\gamma k^+_{ER}}, \tag{31}$$

$$L_{RO} = \frac{L_R}{\omega} = \frac{k^-_{ER} + k^+_{EI1}}{\omega k^+_{ER}}. \tag{32}$$

This analytic expression for the initial catalytic rate can be used to relate experimentally accessible quantities with the elementary rate constants applied in the model or simple combinations thereof. The maximal catalytic rate for carboxylation is given when both ribulose-P_2 and CO_2 are present in excess,

$$v_{\max} = \lim_{[RuBP]\to\infty,[CO_2]\to\infty} v_i = k_{cat}E^{\mathrm{tot}}. \tag{33}$$

K_M-values are experimentally determined under the condition that the other substrate is present in excess. Thus,

$$\lim_{[RuBP]\to\infty} v_i = \frac{v_{\max}[CO_2]}{[CO_2] + \frac{L_{RC}}{L_R} + \frac{L_{RC}}{L_{RO}}\cdot[O_2]} = \frac{v_{\max}[CO_2]}{[CO_2] + K_{M(CO_2)}} \tag{34}$$

with

$$K_{M(CO_2)} = \frac{1}{\gamma}(1 + \omega[O_2]). \tag{35}$$

This expression correctly reflects the observation that different $K_{M(CO_2)}$-values are obtained experimentally for different external oxygen levels. In particular, it is predicted that the K_M-values for aerobic conditions must be larger than those obtained for anaerobic conditions.
Similary,

$$\lim_{[CO_2]\to\infty} v_i = \frac{v_{\max}[RuBP]}{[RuBP]+\frac{L_{RC}}{L_C}} = \frac{v_{\max}[RuBP]}{[RuBP]+K_{M(\mathrm{RuBP})}} \tag{36}$$

with

$$K_{M(\mathrm{RuBP})} = \frac{k_{cat}}{k_{ER}^{+}}. \tag{37}$$

To find a theoretical expression for the Michaelis constant $K_{M(O_2)}$ for oxygen, one has to consider the initial rate w_i of oxygenation, which is determined in analogy to Eq. 27 describing the initial carboxylation rate v_i

$$w_i = k_{oxy}[ERO]_i = k_{oxy}\omega[O_2][ER]_i = \frac{\omega k_{oxy}\frac{k_{ER}^{+}}{k_{ER}^{-}+k_6^{+}}E^{\mathrm{tot}}[RuBP][O_2]}{1+\frac{[RuBP]}{L_R}+\frac{[CO_2]}{L_C}+\frac{[O_2]}{L_O}+\frac{[RuBP][CO_2]}{L_{RC}}+\frac{[RuBP][O_2]}{L_{RO}}} \tag{38}$$

to arrive at

$$\lim_{[RuBP]\to\infty} w_i = \frac{k_{oxy}E^{\mathrm{tot}}[O_2]}{[O_2]+\frac{1}{\omega}(1+\gamma[CO_2])} = \frac{w_{\max}[O_2]}{[O_2]+K_{M(O_2)}} \tag{39}$$

with

$$w_{\max} = k_{oxy}E^{\mathrm{tot}} \quad \text{and} \quad K_{M(O_2)} = \frac{1}{\omega}(1+\gamma[CO_2]). \tag{40}$$

The CO_2 versus O_2 substrate specificity, denoted Ω, is defined as the ratio of the carboxylation rate versus oxygenation rate under the condition that CO_2 and O_2 are present in equal concentrations. This results in the theoretical expression

$$\Omega = \left(\frac{v_i}{w_i}\right)_{[CO_2]=[O_2]} = \frac{\gamma k_{cat}}{\omega k_{oxy}}. \tag{41}$$

Assume, for example, that $V_{\max}$, $K_{M(\mathrm{RuBP})}$, $K_{M(CO_2)}$, $K_{M(O_2)}$ and Ω have been experimentally determined. This set of parameters is in fact available for RuBisCOs from several species. Moreover, the Michaelis constant for CO_2 is usually measured under oxygen-free conditions and that for oxygen under very low CO_2 concentrations. Then, parameters can be determined by resolving Eqs. 33, 35, 37, 40 and 41, to yield

$$k_{cat} = V_{\max}/\text{total enyzme} \tag{42}$$
$$\gamma = 1/K_{M(CO_2)} \tag{43}$$
$$\omega = 1/K_{M(O_2)} \tag{44}$$
$$k_{oxy} = \frac{1}{\Omega}\frac{\gamma}{\omega}\cdot k_{cat} \tag{45}$$
$$k_{ER}^{+} = \frac{k_{cat}}{K_{M(\mathrm{RuBP})}}. \tag{46}$$

If $K_{M(O_2)}$ is not available, but $K_{M(CO_2)}$ has been determined under aerobic and anaerobic conditions, then ω can be estimated by applying Eq. 35 for the two different Michaelis constants:

$$\omega = \frac{1}{[O_2]}\left(\frac{K_{M(CO_2)}^{\mathrm{air}}}{K_{M(CO_2)}}-1\right), \tag{47}$$

where $K_{M(CO_2)}^{\mathrm{air}}$ denotes the value determined under aerobic conditions and $[O_2]$ is the concentration of dissolved oxygen under standard atmospheric conditions ($[O_2]\approx 250\mu M$).
Formulas 42–47 were used to derive the parameters for the fast reactions in the main article.

S2.2 Slow reaction dynamics

Inserting the quasi steady state concentrations for ER and ERO (Eqs. 17 and 19) into the rate expressions for v_{EI1} and v_{EI2} (Eqs. 11 and 12) and assuming that the concentration of free inhibitors are negligible ($[XuBP] = [PDBP] = 0$), transforms Eqs. 4 and 5 describing the slow dynamics of the enzyme-inhibitor complexes into

$$\frac{d[EI1]}{dt} = k^+_{EI1}[ER]_i - (ak^+_{EI1} + k^-_{EI1} + k^-_X)[EI1] - bk^+_{EI1}[EI2], \tag{48}$$

$$\frac{d[EI2]}{dt} = k^+_{EI2}\omega[O_2][ER]_i - ak^+_{EI2}\omega[O_2][EI1] - \left(k^+_{EI2}(b\omega[O_2] - \kappa) + k^-_{EI2} + k^-_P\right)[EI2]. \tag{49}$$

This is a system of two coupled linear differential equations which is analytically solvable.
For large times, the system reaches a steady state, which can simplest be obtained by solving the algebraic equation system resulting from setting all system equations 1–5 to zero:

$$d[EI1]/dt = 0 \Leftrightarrow \overline{[EI1]} = \frac{k^+_{EI1}}{k^-_{EI1} + k^-_X}\overline{[ER]} = \Gamma_1\,\overline{[ER]} \tag{50}$$

$$d[EI2]/dt = 0 \Leftrightarrow \overline{[EI2]} = \frac{k^+_{EI2}}{k^-_{EI2} + k^-_P}\overline{[ERO]} = \Gamma_2\,\overline{[ERO]} \tag{51}$$

$$d[ERO]/dt = 0 \Leftrightarrow \overline{[ERO]} = \frac{k^+_{ERO}}{k^-_{ERO} + \Gamma_2 k^-_P + k_{oxy}}[O_2]\overline{[ER]} = \tilde{\omega}[O_2]\overline{[ER]}. \tag{52}$$

Here, the new abbreviations

$$\Gamma_1 = \frac{k^+_{EI1}}{k^-_{EI1} + k^-_X}, \tag{53}$$

$$\Gamma_2 = \frac{k^+_{EI2}}{k^-_{EI2} + k^-_P}, \tag{54}$$

$$\tilde{\omega} = \frac{k^+_{ERO}}{k^-_{ERO} + \Gamma_2 k^-_P + k_{oxy}} \tag{55}$$

have been introduced. Inserting Eq. 51 into Eq. 19 and comparing with Eq. 52 yields the relation

$$\omega = \tilde{\omega}(1 - \kappa\Gamma_2). \tag{56}$$

The parameter k^-_{EI2} describes the rate constant for the back transformation of the ihibitor PDBP, which is in principle possible but requires the binding of free H_2O_2. Therefore, under *in-vivo* as well as *in-vitro* conditions, this rate can be considered to be extremely small. As a result, $\kappa \ll 1$ and to a very good approximation, $\omega \approx \tilde{\omega}$.
To determine the final catalysis rate, the steady state concentration of the enzyme-substrate complex ER has to be determined. Setting Eq. 1 to zero and considering the conservation rule Eq. 15 yields with the above results

$$\overline{[ER]} = \frac{k^+_{ER}[RuBP]E^{\mathrm{tot}}}{k^+_{ER}[RuBP]\,(1 + \gamma[CO_2] + \Gamma_1 + (1+\Gamma_2)\tilde{\omega}[O_2]) + k^-_{ER} + k_{cat}\gamma[CO_2] + (k_{oxy} + \Gamma_2 k^-_P)\tilde{\omega}[O_2] + \Gamma_1 k^-_X}. \tag{57}$$

The final rate of catalysis is therefore given by

$$v_f = k_{cat}\gamma[CO_2]\overline{[ER]}, \tag{58}$$

from which the analytic expression of the fallover extent follows:

$$f = 1 - \frac{v_f}{v_i} = 1 - \frac{\overline{[ER]}}{[ER]_i} \tag{59}$$

$$= \frac{k^+_{ER}[RuBP]\,(\Gamma_1 + ((1+\Gamma_2)\tilde{\omega} - \omega)\,[O_2]) + \left((k_{oxy} + \Gamma_2 k^-_P)\tilde{\omega} - (k_{oxy} + k^+_{EI2})\omega\right)[O_2] + \Gamma_1 k^-_X - k^+_{EI1}}{k^+_{ER}[RuBP]\,(1 + \gamma[CO_2] + \Gamma_1 + (1+\Gamma_2)\tilde{\omega}[O_2]) + k^-_{ER} + k_{cat}\gamma[CO_2] + (k_{oxy} + \Gamma_2 k^-_P)\tilde{\omega}[O_2] + \Gamma_1 k^-_X}. \tag{60}$$

This expression can be considerably simplified by employing some plausible approximations. First, production of inhibitor is slow compared to the reactions in the main catalytic pathways. In particular, $\Gamma_1 k^-_X \ll k^+_{ER}[RuBP]$ and $k^+_{EI1} \ll k^+_{ER}[RuBP]$ allows to ignore the corresponding terms in the numerator and the denominator. Further, $\left((k_{oxy} + \Gamma_2 k^-_P)\tilde{\omega} - (k_{oxy} + k^+_{EI2})\omega\right) \approx 0$, since

$\omega \approx \tilde{\omega}$ and, due to the separation of time scales, $k^+_{EI2} \ll k_{oxy}$ and $\Gamma_2 k^-_P \ll k_{oxy}$. Lastly, under the experimental as well as under *in-vivo* conditions, the concntration of RuBP is large, therefore $k^-_{ER} \ll k^+_{ER}[RuBP]$. These deliberations lead to the approximate expression of the fallover extent

$$f \approx \frac{k^+_{ER}[RuBP](\Gamma_1 + \Gamma_2\omega[O_2])}{k^+_{ER}[RuBP]\,(1+\gamma[CO_2]+\Gamma_1+(1+\Gamma_2)\omega[O_2]) + k_{cat}\gamma[CO_2] + k_{oxy}\omega[O_2]} \tag{61}$$

In anaerobic conditions ($[O_2] = 0$), this expression further simplifies to

$$f^{\text{anaer}} \approx \frac{k^+_{ER}[RuBP]\Gamma_1}{k^+_{ER}[RuBP]\,(1+\gamma[CO_2]+\Gamma_1) + k_{cat}\gamma[CO_2]}. \tag{62}$$

Taking into account relations 35 and 37,

$$f^{\text{anaer}} \approx \frac{\Gamma_1}{1 + \frac{[CO_2]}{K_{M(CO_2)}} + \Gamma_1 + \frac{[CO_2]}{K_{M(CO_2)}} \cdot \frac{K_{M(\text{RuBP})}}{[RuBP]}}, \tag{63}$$

which allows to estimate Γ_1 from experimentally determined fallover extents under anaerobic conditions by

$$\Gamma_1 \approx x \cdot \frac{f^{\text{anaer}}}{1 - f^{\text{anaer}}} \quad \text{with} \quad x = 1 + \frac{[CO_2]}{K_{M(CO_2)}}\left(1 + \frac{K_{M(\text{RuBP})}}{[RuBP]}\right). \tag{64}$$

After estimation of Γ_1, experimentally determined fallover extents under aerobic conditions can be used, employing Eqs. 61, 40 and 41, to reach an estimation of Γ_2 by

$$\Gamma_2 \approx \frac{yf - z}{1 - f} \quad \text{with} \quad y = \frac{K_{M(O_2)}}{[O_2]}(x + \Gamma_1) + 1 + \frac{1}{\Omega}\frac{K_{M(O_2)}}{K_{M(CO_2)}}\frac{K_{M(\text{RuBP})}}{[RuBP]} \quad \text{and} \quad z = \frac{K_{M(O_2)}}{[O_2]}\Gamma_1. \tag{65}$$

To exploit experimental data on the characteristic fallover times, the linear differential equation system given by Eqs. 48 and 49 has to be solved. This system can compactly be written as

$$\frac{dw}{dt} = Aw + \zeta, \tag{66}$$

where

$$w = \begin{pmatrix} [EI1] \\ [EI2] \end{pmatrix}, \quad A = -\begin{pmatrix} ak^+_{EI1} + k^-_{EI1} + k^-_X & bk^+_{EI1} \\ ak^+_{EI2}\omega[O_2] & k^+_{EI2}(b\omega[O_2] - \kappa) + k^-_{EI2} + k^-_P \end{pmatrix} \quad \text{and} \quad \zeta = \begin{pmatrix} k^+_{EI1}[ER]_i \\ k^+_{EI2}\omega[O_2][ER]_i \end{pmatrix}. \tag{67}$$

Introducing

$$v = w + A^{-1}\zeta, \tag{68}$$

transforms the inhomogeneous into a homogeneous system

$$\frac{dv}{dt} = A \cdot v, \tag{69}$$

which has the general solution

$$v(t) = c_1 \cdot u_1 \cdot e^{\lambda_1 t} + c_2 \cdot u_2 \cdot e^{\lambda_2 t}, \tag{70}$$

where $\lambda_{1/2}$ are the eigenvalues of the matrix A and $u_{1/2}$ the corresponding eigenvectors. The real constants $c_{1/2}$ have to be determined from the initial condition

$$v(0) = c_1 \cdot u_1 + c_2 \cdot u_2 = A^{-1}\zeta, \tag{71}$$

which follows from $w(0) = 0$. This translates back to

$$[EI1](t) = c_1 u^{(1)}_1 e^{\lambda_1 t} + c_2 u^{(1)}_2 e^{\lambda_2 t} + \overline{[EI1]} \tag{72}$$

$$[EI2](t) = c_1 u^{(2)}_1 e^{\lambda_1 t} + c_2 u^{(2)}_2 e^{\lambda_2 t} + \overline{[EI2]} \tag{73}$$

and

$$[ER](t) = [ER]_i - a\overline{[EI1]} - b\overline{[EI2]} - c_1(au^{(1)}_1 + bu^{(2)}_1)e^{\lambda_1 t} - c_2(au^{(1)}_2 + bu^{(2)}_2)e^{\lambda_2 t}. \tag{74}$$

In the anaerobic case, the equation system reduces to the single equation given by Eq. 48 and the eigenvalue is simply given by

$$-\lambda = ak_{EI1}^{+} + k_{EI1}^{-} + k_{X}^{-} = k_{\text{obs}}^{\text{anaer}}, \tag{75}$$

with its absolute value equalling the observed characterstic fallover time $k_{\text{obs}}^{\text{anaer}}$ under anaerobic conditions. Together with the estimated value of Γ_1 (see Eq. 53), this relation allows to provide reasonable estimates for the single rates k_{EI1}^{+}, k_{EI1}^{-} and k_{X}^{-}.
To exploit the observed characteristic fallover time in the aerobic case is more difficult. Since in the original publications the fallover time-courses were plotted on linear scale, it was difficult to detect two time constants in the activity decline. In the fit to obtain k_{obs}, we therefore assume that in fact a single exponential function was fitted to a superposition of two declining exponentials. Mathematically, this corresponds to choosing a value k, such that the difference between the functions

$$e^{-kt} \quad \text{and} \quad \alpha e^{-\lambda t} + (1-\alpha)e^{-\mu t} \tag{76}$$

is minimized. Here, k corresponds to the experimentally observed characteristic fallover time, $\lambda = -\lambda_1$ and $\mu = -\lambda_2$ to the negative eigenvalues and α describes the weights with which the two exponentials are summed. From Eq. 74 it follows that

$$\alpha = \frac{c_1(au_1^{(1)} + bu_1^{(2)})}{c_1(au_1^{(1)} + bu_1^{(2)}) + c_2(au_2^{(1)} + bu_2^{(2)})}. \tag{77}$$

For the theoretical prediciton of the observed characteristic fallover time k, the integral of the mean square residuals was minimized. This is given by

$$r(k) = \int_0^{\infty} \left(\alpha e^{-\lambda t} + (1-\alpha)e^{-\mu t} - e^{-kt}\right)^2 dt = \text{const} + \frac{1}{2k} - \frac{2a}{\lambda + k} - \frac{2(1-a)}{\mu + k}. \tag{78}$$

A necessary condition for the minimum is given by

$$\frac{dr}{dk} = 0 \Leftrightarrow 3k^4 + k^3\left(8\alpha\mu + 8(1-\alpha)\lambda - 2\lambda - 2\mu\right) + k^2\left(4\alpha\mu + 4(1-\alpha)\lambda - \lambda^2 - \mu^2 - 4\lambda\mu\right) - k\left(2\lambda\mu(\lambda+\mu)\right) - \lambda^2\mu^2 = 0. \tag{79}$$

The only positive root of this quartic equation was determined numerically for all plots in which theoretical predictions of the observed characteristic fallover times are given.

S3 Model equations for the extended model

For the more detailed, represented in Fig. 6 in the main text, the ten mass balance equations read

$$d[ER]/dt = v_{ER} - v_{EE1} \tag{80}$$
$$d[EE1]/dt = v_{EE1} - v_{ERC} - v_{ERO} - v_{SW} - v_{EDP1} \tag{81}$$
$$d[EE2]/dt = v_{EE2} + v_{SW} - v_{EDP2} \tag{82}$$
$$d[EDP1]/dt = v_{EDP1} - v_{D1} \tag{83}$$
$$d[EDP2]/dt = v_{EDP2} - v_{D2} \tag{84}$$
$$d[ERC]/dt = v_{ERC} - v_{\text{cat}} \tag{85}$$
$$d[ERO]/dt = v_{ERO} - v_{\text{oxy}} - v_{EI2} \tag{86}$$
$$d[EI1]/dt = v_{EI1} - v_{EE2} \tag{87}$$
$$d[EI2]/dt = v_{EI2} + v_{P} \tag{88}$$
$$d[RuBP]/dt = -v_{ER}. \tag{89}$$

In contrast to model 1, the concentration of the substrate RuBP is now considered to be a variable. For simulations in which a large amount of RuBP is applied initially, this change is negligible. However, for simulations in which only XuBP is initially available, the slow release of RuBP and the according equilibriation process is crucial to explain the apparent increase in catalytic activity of the Val-335 mutant on XuBP as substrate. The substrate concentrations of XuBP, CO_2 and O_2 are considered to be fixed.

The rate laws are again derived from simple mass-action kinetic rate laws reflecting the elementary processes depicted in Fig. 6 of the main text:

$$v_{ER} = k_{ER}^{+}[E][RuBP] - k_{ER}^{-}[ER] \tag{90}$$
$$v_{X} = k_{X}^{+}[E][XuBP] - k_{X}^{-}[EI1] \tag{91}$$
$$v_{SW} = k_{SW}^{+}[EE1] - k_{SW}^{-}[EE2] \tag{92}$$
$$v_{ERC} = k_{ERC}^{+}[EE1][CO_2] - k_{ERC}^{-}[ERC] \tag{93}$$
$$v_{ERO} = k_{ERO}^{+}[EE1][O_2] - k_{ERO}^{-}[ERO] \tag{94}$$
$$v_{EI2} = k_{EI2}^{+}[ERO] - k_{EI2}^{-}[EI2] \tag{95}$$
$$v_{cat} = k_{cat}[ERC] \tag{96}$$
$$v_{oxy} = k_{oxy}[ERO] \tag{97}$$
$$v_{P} = k_{P}^{+}[E][PDBP] - k_{P}^{-}[EI2] \tag{98}$$
$$v_{EE1} = k_{EE1}^{+}[ER] - k_{EE1}^{-}[EE1] \tag{99}$$
$$v_{EE2} = k_{EE2}^{+}[EI1] - k_{EE2}^{-}[EE2] \tag{100}$$
$$v_{EDP1} = k_{EDP1}[EE1] \tag{101}$$
$$v_{EDP2} = k_{EDP2}[EE2] \tag{102}$$
$$v_{D1} = k_{D1}[EDP1] \tag{103}$$
$$v_{D2} = k_{D2}[EDP2] \tag{104}$$

In analogy to Eq. 15, the conservation relation involving all enzyme-containing species reads

$$[E] + [ER] + [EE1] + [EE2] + [EDP1] + [EDP2] + [ERC] + [ERO] + [EI1] + [EI2] = E^{\mathrm{tot}}, \tag{105}$$

and is used to eliminate the variable $[E]$ for the free enzyme concentration.
The kinetic simulations were carried out using the MATLAB solver for stiff differential equations `ode15s`.

S4 Summation theorems for response coefficients

All response coefficients were determined numerically by changing each parameter by a small amount and recording the changes in the observed quantity. The definition of the response coefficient of a given parameter p on an output quantity X is defined by

$$R_p^X = \frac{\partial \ln X}{\partial \ln p}. \tag{106}$$

If all parameters are increased simultaneously by a factor λ, all rates must increase also by the factor of λ, and all characteristic times must be reduced by a factor λ. The fallover extent, which is the ratio of two rates, is unaffected. Mathematically, if $f = f(k_1, \ldots k_m)$ defines the fallover extent as a function of all rate parameters k_i,

$$f(\lambda k_1, \ldots \lambda k_m) = f(k_1, \ldots, k_m) \tag{107}$$

is a homogeneous function of order 0. Thus, the summation theorem

$$\sum_i R_{k_i}^f = 0 \tag{108}$$

is a direct consequence of Euler's theorem on homogeneous functions.
Similarly,

$$v_{\max}(\lambda k_1, \ldots \lambda k_m) = \lambda v_{\max}(k_1, \ldots, k_m) \tag{109}$$

implies

$$\sum_i R_{k_i}^{v_{\max}} = 1 \tag{110}$$

and

$$T_{\max}(\lambda k_1, \ldots \lambda k_m) = \lambda^{-1} T_{\max}(k_1, \ldots, k_m) \tag{111}$$

implies

$$\sum_i R_{k_i}^{T_{\max}} = -1. \tag{112}$$

The numerically determined response coefficients are given in Tables S1, S3 and S4. For these, all summation theorems are fulfilled with extremely good accuracy.

Table S1. Response coefficients for fallover extent and characteristic time under anaerobic and aerobic conditions.

	no oxygen		atmospheric oxygen	
Parameter	f	k_{obs}	f	k_{obs}
k_{ER}^{+}	0.03	0.01	0.01	0.03
k_{ER}^{-}	0	0	0	0
k_{ERC}^{+}	-0.72	-0.24	-0.32	-0.64
k_{ERC}^{-}	0	0	0	0
k_{ERO}^{+}	–	–	0.27	0.39
k_{ERO}^{-}	–	–	0	-0.01
k_{cat}	0.69	0.23	0.30	0.62
k_{oxy}	–	–	-0.27	-0.37
k_{EI1}^{+}	0.75	0.25	0.06	0.28
k_{EI1}^{-}	-0.75	0.75	-0.06	-0.14
k_{EI2}^{+}	–	–	0.28	0.40
k_{P}^{-}	–	–	-0.28	0.45

Table S2. Parameters for wild-type and Val-335 RuBisCO from tobacco for the extended model. These parameter were used to simulate wildtype RuBisCO and the Val-335 mutant with the extended model, with which in particular the bahaviour on XuBP as substrate were investigated. Those parameters for the wild-type with analogues in the simple model were largely left unchanged. The remaining parameters were fitted manually in order to quantitatively reflect the main experimental results on XuBP carboxylation from the experimental publications cited in the main text. While fitting the parameters, it was ensured that the original model behavior (fallover on RuBP as substrate) were not altered. Parameters for the Val-335 mutants were reasoned in a similar fashion as described for model parameters for the simple models and manually fitted to reproduce experimental data.

Parameter	wildtype	Val-335
k_{ER}^{+}	0.15 $\mu\mathrm{M}^{-1}$ s^{-1}	0.01 $\mu\mathrm{M}^{-1}$ s^{-1}
k_{ER}^{-}	0.048 s^{-1}	5 s^{-1}
k_{EE1}^{+}	100 s^{-1}	2 s^{-1}
k_{EE1}^{-}	1 s^{-1}	6 s^{-1}
k_{ERC}^{+}	0.302 $\mu\mathrm{M}^{-1}$ s^{-1}	0.052 $\mu\mathrm{M}^{-1}$ s^{-1}
k_{ERC}^{-}	0.02 s^{-1}	0.02 s^{-1}
k_{ERO}^{+}	0.0012 $\mu\mathrm{M}^{-1}$ s^{-1}	6.45×10^{-4} $\mu\mathrm{M}^{-1}$ s^{-1}
k_{ERO}^{-}	0.02 s^{-1}	0.02 s^{-1}
k_{cat}	3.1 s^{-1}	3 s^{-1}
k_{oxy}	1.125 s^{-1}	0.625 s^{-1}
k_{SW}^{+}	0.02 s^{-1}	0.0162 s^{-1}
k_{SW}^{-}	9×10^{-4} s^{-1}	0.023 s^{-1}
k_{EDP1}	0 s^{-1}	0.001 s^{-1}
k_{D1}	0 s^{-1}	1 s^{-1}
k_{X}^{+}	0.015 $\mu\mathrm{M}^{-1}$ s^{-1}	0.005 $\mu\mathrm{M}^{-1}$ s^{-1}
k_{X}^{-}	0.075 s^{-1}	2.5 s^{-1}
k_{EE2}^{+}	0.01 s^{-1}	5 s^{-1}
k_{EE2}^{-}	5×10^{-4} s^{-1}	10 s^{-1}
k_{EDP2}	0.003 s^{-1}	0.001 s^{-1}
k_{D2}	0.002 s^{-1}	1 s^{-1}
k_{EI2}^{+}	0.1 s^{-1}	0.028 s^{-1}
k_{EI2}^{-}	0 s^{-1}	2.85×10^{-5} s^{-1}
k_{P}^{+}	0 $\mu\mathrm{M}^{-1}$ s^{-1}	0 $\mu\mathrm{M}^{-1}$ s^{-1}
k_{P}^{-}	5.5×10^{-4} s^{-1}	0 s^{-1}

Table S3. Response coefficients for fallover extent and characteristic time on XuBP as substrate for wildtype RuBisCO.

Parameter	$v_{\max}$	$T_{\max}$	f	$T_{1/2}$
k_X^+	0.0344	-0.0605	0.0288	-0.0424
k_X^-	-0.0331	0.0543	-0.0296	0.0306
k_{EE2}^+	0.3617	-0.5964	0.3211	-0.3381
k_{EE2}^-	-0.0278	-0.0151	-0.0150	0.0036
k_{SW}^+	0.9492	-0.0277	-0.0268	0.0068
k_{SW}^-	-0.0002	0.0000	0.0000	-0.0000
k_{ER}^+	0.0000	0.0000	-0.0002	-0.0002
k_{ER}^-	-0.0000	0.0000	-0.0000	-0.0000
k_{EE1}^+	0.0000	-0.0000	0.0000	0.0000
k_{EE1}^-	-0.0000	0.0000	-0.0000	-0.0000
k_{ERC}^+	0.0125	0.0000	-0.0007	-0.0002
k_{ERC}^-	-0.0001	-0.0000	0.0000	0.0000
k_{ERO}^+	-0.0123	-0.0001	0.0007	0.0003
k_{ERO}^-	0.0002	0.0000	-0.0000	-0.0000
k_{EI2}^+	-0.0001	-0.0001	0.0006	0.0002
k_{EI2}^-	0	0	0	0
k_{EDP1}	0	0	0	0
k_{EDP2}	-0.2992	-0.4126	0.5042	-0.3495
k_{D1}	0	0	0	0
k_{D2}	0.0145	0.0597	-0.7819	-0.3090
k_{cat}	0.0002	-0.0017	0.0001	0.0000
k_{oxy}	-0.0001	0.0001	-0.0006	-0.0002

Table S4. Response coefficients for fallover extent and characteristic time on XuBP as substrate for the Val-335 mutant.

Parameter	v^*	T^*	f	$T_{1/2}$
k_X^+	0.84	-0.13	0.07	-0.03
k_X^-	-0.99	-0.75	0.38	-0.82
k_{EE2}^+	0.99	0.26	-0.13	0.27
k_{EE2}^-	-1.01	-0.36	0.01	-0.34
k_{SW}^+	0.98	-0.02	0.01	-0.04
k_{SW}^-	-0.06	0	0	0
k_{ER}^+	0	0	0.01	-0.95
k_{ER}^-	-0.06	0	0.16	1.05
k_{EE1}^+	0.07	0	-0.18	-1.01
k_{EE1}^-	-0.24	0	0.70	1.11
k_{ERC}^+	0.28	0.01	-0.75	-0.97
k_{ERC}^-	0	0	0	0.01
k_{ERO}^+	-0.01	0.01	0	-0.04
k_{ERO}^-	0	0	0	0
k_{EI2}^+	0	-0.01	0.01	-0.03
k_{EI2}^-	0	0	0	0
k_{EDP1}	0	0	0	0
k_{EDP2}	0	0	0	0
k_{D1}	0	0	0	0
k_{D2}	0	0	0	0
k_{cat}	0.15	0	-0.42	0.79
k_{oxy}	0	0.01	-0.01	0.03