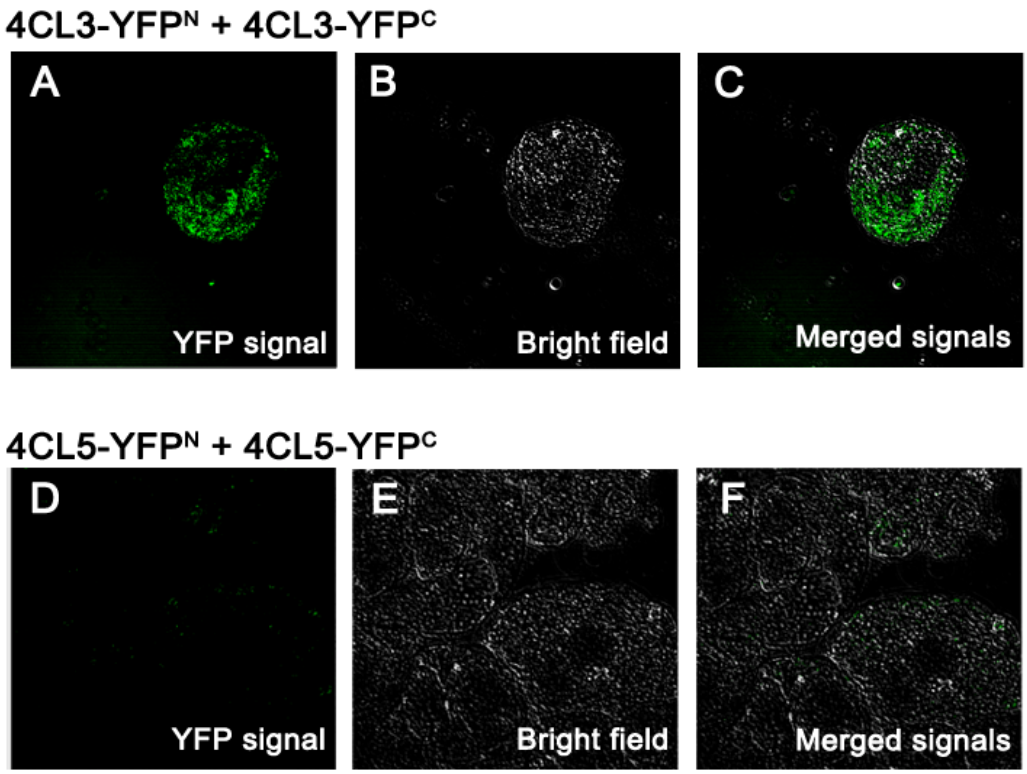
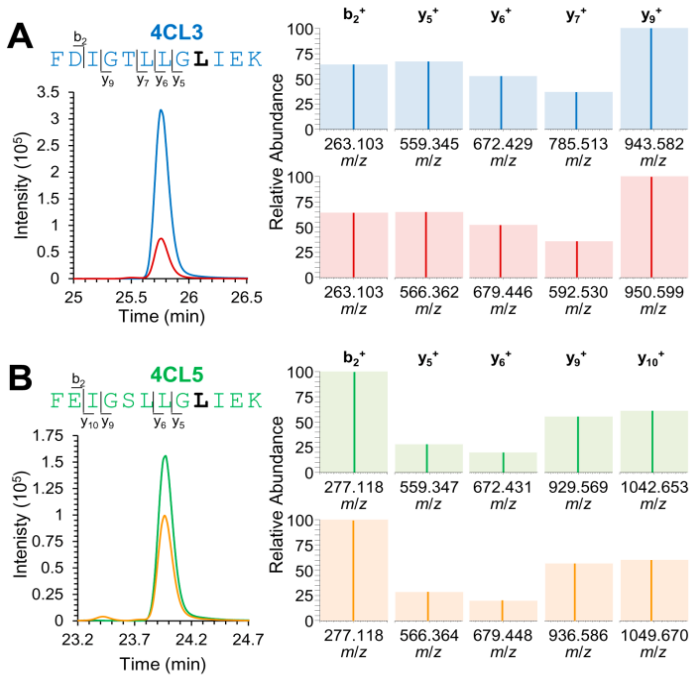


Supplemental Figure 1. Bimolecular Fluorescence Complementation (BiFC) of homomeric *P. trichocarpa* 4CL3 and 4CL5.

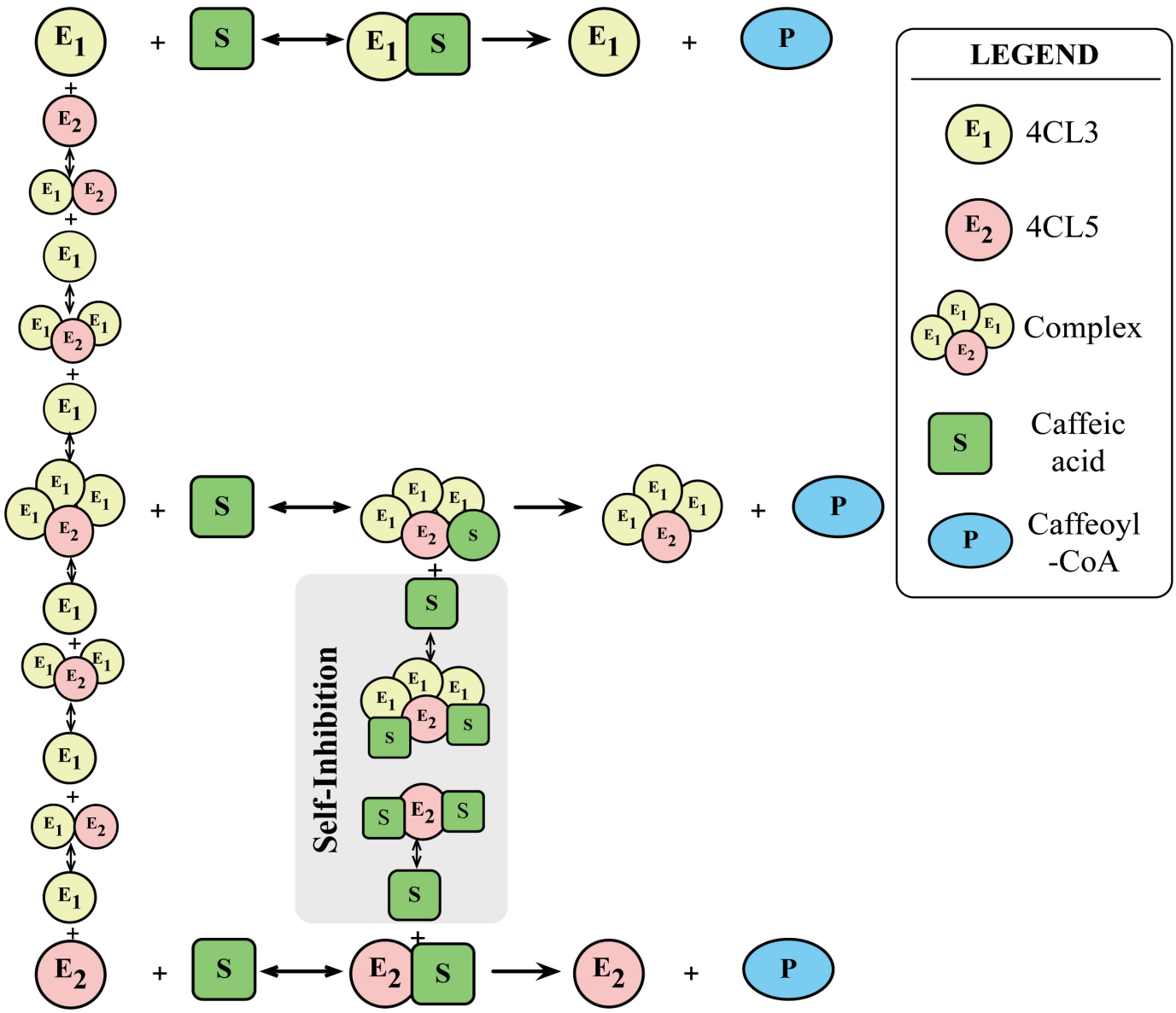
4CL fusion proteins of N terminal or C terminal fragments of YFP. (A) to (C) 4CL3-YFP^N + 4CL3-YFP^C and (D) to (F) 4CL5-YFP^N + 4CL5-YFP^C .



Supplemental Figure 2. Quantification of the 4CL3 and 4CL5 proteins by PC-IDMS.

Extracted ion chromatograms and associated SRM-mass spectra were measured for the surrogate peptide (blue/green) and corresponding SIL peptide standard (red/orange) for **(A)** 4CL3 and **(B)** 4CL5. All chromatograms represent the LC retention times for the labeled surrogate peptide and the tryptic peptide derived from the sample. The ion intensities of the five fragment ions monitored by SRM for the respective peptide are shown, where the associated mass spectra have a width of 0.002 m/z. The charge to mass ratios of the fragment ions are shown as m/z. The intensity of the peptides is shown as intensity relative to the standard. In A, the blue peak is shown relative to the red standard, while B shows the sample (green) compared to the standard (orange). The position within the peptide of the cleavage products leading to the five specific fragment ions are shown for A and B above the chromatograms. The bold residue in the peptide represents the heavy isotope label of the synthetic peptide standard. Thin lines in the histograms represent signal intensity as fragment relative abundance.

A



B

Equation

$$v = \frac{k_{cat_1}\cdot[E_{1t}][S]}{K_{m_1}+[S]+\frac{3\cdot K_{m_1}\cdot[E_{1t}]^2[E_{2t}]}{k_1^3}\cdot(1+\frac{[S]}{K_{m_2}}+\frac{[S]^2}{K_{m_2}\cdot K_{is}})} + \frac{k_{cat_2}\cdot[E_{2t}][S]\cdot(1+\gamma\cdot(\frac{[E_{1t}]}{k_2})^3)}{(K_{m_2}+[S]+\frac{[S]^2}{K_{is}})\cdot(1+(\frac{[E_{1t}]}{k_2})^3)}$$

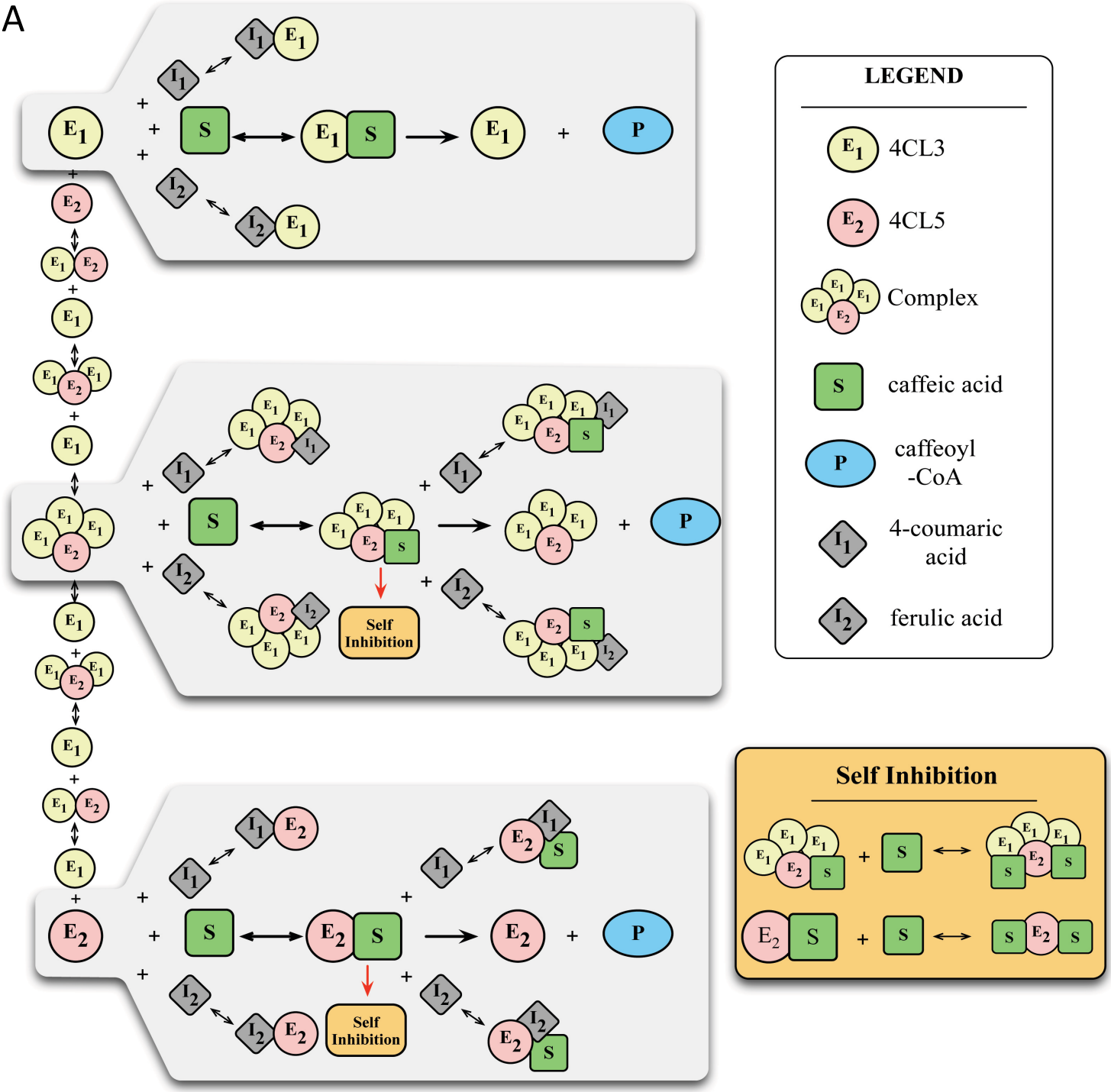
Optimized parameters

k_1	0.024 ± 0.010
k_2	0.054 ± 0.008
γ	5.189 ± 1.067

Supplemental Figure 3. Mechanistic description of the inhibition and activation effects on the rate of product formation using caffeic acid as substrate.

(A) This diagram parallels that of Figure 5B but has caffeic acid as substrate. Caffeic acid has self-inhibition with a rate $1/k_{is}$ which is considered in the 4CL5 and in the 4CL3/4CL5 enzyme complex reactions. Other relationships between components are the same as in Figure 5A and 8A. **(B)** describes the mathematical model for multiple enzymes and single substrate with caffeic acid as substrate. The equation represents the rate of total product formation associated with 4CL3, 4CL5 and the 4CL3/4CL5 complex, where k_{iS} is a self-inhibition kinetic parameter for 4CL5 and the 4CL3/4CL complex. The definitions of other variables and parameters are the same as those in Figure 8B. $k1$, $k2$ and γ values represent the mean ± standard deviation of 100 optimized values.

A



B

Equation

$$v = \frac{k_{cat_1}\cdot[E_{1t}][S]}{K_{m_1}\cdot(1+\frac{[I_1]}{K3_{ic_1}}+\frac{[I_2]}{K3_{ic_2}})+[S]+\frac{3\cdot K_{m_1}\cdot[E_{1t}]^2[E_{2t}]}{k_1^3}\cdot((1+\frac{[I_1]}{K5_{ic_1}}+\frac{[I_2]}{K5_{ic_2}})+\frac{[S]}{K_{m_2}}\cdot(1+\frac{[I_1]}{K5_{iu_1}}+\frac{[I_2]}{K5_{iu_2}}+\frac{[S]}{K_{is}}))}$$

$$+\frac{k_{cat_2}\cdot[E_{2t}][S]\cdot(1+\gamma\cdot(\frac{[E_{1t}]}{k_2})^3)}{(K_{m_2}\cdot(1+\frac{[I_1]}{K5_{ic_1}}+\frac{[I_2]}{K5_{ic_2}})+[S]\cdot(1+\frac{[I_1]}{K5_{iu_1}}+\frac{[I_2]}{K5_{iu_2}}+\frac{[S]}{K_{is}}))\cdot(1+(\frac{[E_{1t}]}{k_2})^3)}$$

Optimized parameters

k_1	0.026 ± 0.007
k_2	0.072 ± 0.014
γ	3.381 ± 1.106

Supplemental Figure 4. Mechanistic description and mathematical model including the multiple inhibition effects on product formation with caffeic acid as the main substrate.

(A) Self-inhibition effects with a rate $1/k_{is}$ are considered in the 4CL5 and 4CL3/4CL5 complex enzymatic reactions. Other relationships between components are the same as in Figure 8A. We assume that the enzyme complex takes on the same characteristics due to the dominance of the 4CL5. The figure illustrates the mechanistic configuration for substrate self-inhibition of caffeic acid in the presence of 4-coumaric acid and ferulic acid. Again, k_{is} is the self-inhibition binding rate for both E2 (4CL5) and the enzyme complex. **(B)** A mathematical model for multiple enzymes and multiple substrates, with caffeic acid as the main substrate, and with 4-coumaric acid and ferulic acid as inhibitors. The equation represents the rate of total product formation associated with 4CL3, 4CL5 and the 4CL3/4CL5 complex, where k_{iS} is a self-inhibition kinetic parameter of 4CL5 and the 4CL3/4CL complex and the definitions of other variables and parameters are the same as those in $k1$, $k2$ and γ values represent the mean ± standard deviation of 100 optimized values.

Supplemental Method

1. Mathematical Model Definitions, Details and Kinetic Parameters

I. Mathematical model for the rate of production formation using 4CL3 and 4CL5 as multiple enzymes, and 4-coumaric acid as a single substrate

- Equation - Figure 5C
- Variables
  [E1t] : 4CL3 total enzyme concentration (0nM – 40nM)
  [E2t] : 4CL5 total enzyme concentration (0nM – 40nM)
  [S] : 4-coumatic acid substrate concentration
  (28.28uM, 37.97uM, 50.625uM, 67.5uM, 90uM, 160uM)
- Kinetic Constants
  K_{m_1}: Michaelis-Menten constant for 4CL3 enzyme (47.04uM)
  K_{m_2}: Michaelis-Menten constant for 4CL5 enzyme (19.029uM)
  k_{cat_1}: Product rate constant for 4CL3 enzyme (15.267min^{-1})
  k_{cat_2}: Product rate constant for 4CL5 enzyme (22.272min^{-1})
- Optimized parameters
  $k_1,\ k_2,\ \gamma$: Figure 5C

II. Mathematical model for the rate of production formation using 4CL3 and 4CL5 as multiple enzymes, and caffeic acid as a single substrate

- Equation – Supplemental Figure 3B online
- Variables
  [E1t] : 4CL3 total enzyme concentration (0nM – 40nM)
  [E2t] : 4CL5 total enzyme concentration (0nM – 40nM)
  [S] : 4-coumatic acid substrate concentration
  (5.93uM, 7.91uM, 10.55uM, 14.06uM, 18.75uM, 25uM)
- Kinetic Constants
  K_{m_1}: Michaelis-Menten constant for 4CL3 enzyme (5.558 uM)
  K_{m_2}: Michaelis-Menten constant for 4CL5 enzyme (9 uM)
  k_{cat_1}: Product rate constant for 4CL3 enzyme (14.045 min^{-1})
  k_{cat_2}: Product rate constant for 4CL5 enzyme (14 min^{-1})
  k_{is} : Caffeic acid self-inhibition constant for 4CL5 enzyme (55.97uM)
- Optimized parameters
  $k_1,\ k_2,\ \gamma$: Supplemental Figure 3B online

III. Mathematical model for the rate of production formation using 4CL3 and 4CL5 as multiple enzymes, 4-coumaric acid as a main substrate, and caffeic acid, and ferulic acid as inhibitors.

- Equation - Figure 8B
- Variables
  [E1t] : 4CL3 total enzyme concentration (0nM – 40nM)
  [E2t] : 4CL5 total enzyme concentration (0nM – 40nM)
  [S] : 4-coumatic acid substrate concentration
  (17.8uM, 23.73uM, 31.64uM, 42.19uM, 100uM)
- Kinetic Constants
  K_{m_1}: Michaelis-Menten constant for 4CL3 enzyme (18 uM)
  K_{m_2}: Michaelis-Menten constant for 4CL5 enzyme (83 uM)
  k_{cat_1}: Product rate constant for 4CL3 enzyme (54 min^{-1})
  k_{cat_2}: Product rate constant for 4CL5 enzyme (175 min^{-1})
  $K3_{ic_1}$: Caffeic acid competitive inhibition constants for 4CL3 (9.22 uM)
  $K3_{ic_2}$: Ferulic acid competitive inhibition constants for 4CL3 (55.05 uM)
  $K5_{ic_1}$: Caffeic acid competitive inhibition constants for 4CL5 (7.06 uM)
  $K5_{ic_2}$: Ferulic acid competitive inhibition constants for 4CL5 (335.54 uM)
  $K5_{iu_1}$: Caffeic acid uncompetitive inhibition constants for 4CL5 (49.75 uM)
  $K5_{iu_2}$: Ferulic acid uncompetitive inhibition constants for 4CL5 (178.21 uM)

- Optimized parameters
  $k_1,\ k_2,\ \gamma$: Figure 8B

IV. Mathematical model for the rate of production formation using 4CL3 and 4CL5 as multiple enzymes, caffeic acid as a main substrate, and 4-coumaric acid and ferulic acid as inhibitors.

- Equation – Supplement Figure 4B online
- Variables
  [E1t] : 4CL3 total enzyme concentration (0nM – 40nM)
  [E2t] : 4CL5 total enzyme concentration (0nM – 40nM)
  [S] : 4-coumaric acid substrate concentration
  (23.73uM, 31.64uM, 42.19uM, 56.25uM)
- Kinetic Constants
  K_{m_1}: Michaelis-Menten constant for 4CL3 enzyme (3.53 uM)
  K_{m_2}: Michaelis-Menten constant for 4CL5 enzyme (44.97 uM)
  k_{cat_1}: Product rate constant for 4CL3 enzyme (25.84 min^{-1})

k_{cat_2}: Product rate constant for 4CL5 enzyme (120.38 min^{-1})
$K3_{ic_1}$: 4-coumaric acid competitive inhibition constants for 4CL3 (43.73 uM)
$K3_{ic_2}$: Ferulic acid competitive inhibition constants for 4CL3 (59.36 uM)
$K5_{ic_1}$: 4-coumaric acid competitive inhibition constants for 4CL5 (122.70 uM)
$K5_{ic_2}$: Ferulic acid competitive inhibition constants for 4CL5 (518.93 uM)
$K5_{iu_1}$: 4-coumaric acid uncompetitive inhibition constants for 4CL5 (78.07 uM)
$K5_{iu_2}$: Ferulic acid uncompetitive inhibition constants for 4CL5 (372.06 uM)
k_{is} : Caffeic acid self-inhibition constant for 4CL5 enzyme (55.97 uM)

- Optimized parameters

  k_1, k_2, γ : Supplemental Figure 4B online

2. Model Derivation.

I. Explicit form of Equation 1 ($v_{tot}= v_{4CL3} + v_{4CL5}$) in the text.

$$v_{tot} = \frac{k_{cat_1} \cdot [E1t] \cdot [S]}{K_{m_1} + [S]} + \frac{k_{cat_2} \cdot [E2t] \cdot [S]}{K_{m_2} + [S]}$$

II. Enzymatic Reaction Equation of the Interaction of 4CL3 and 4CL5

A. Diagrams

Figure 5B (4-coumaric acid), Supplemental Figure 3A online (caffeic acid)

B. Assumptions

The enzymatic reactions via Michaelis-Menten kinetics are constructed based on quasi-equilibrium assumptions and the conservation of the total enzyme [1]. Terms, with *, representing the inclusion of self-inhibition in each equation are applied for the model utilizing caffeic acid as the main substrate only. Quasi-equilibrium assumes that the association and disassociation between the components related to enzymes and substrates are in binding equilibrium. Enzyme-substrate complexes are defined as:

$$[E1S]=\frac{1}{K_{m_1}}[E1][S] \qquad (S1)$$

$$[E2S]=\frac{1}{K_{m_2}}[E2][S] \quad (S2)$$

where K_{m_1} and K_{m_2} are the Michaelis constants for 4CL3 and 4CL5, respectively.

The complex $[E2(S)_2]$ considering self-inhibition is written as:

$$[E2(S)_2]^*=\frac{1}{K_{is}}[E2S][S]=\frac{1}{K_{m_2}\cdot K_{is}}[E2][S]^2, \quad (S3)$$

where K_{is} is the self-inhibition constant.

The enzyme-complexes and their intermediates are represented as:

$$[E1E2]=\frac{1}{k}[E1][E2] \quad (S4)$$

$$[(E1)_2E2]=\frac{1}{k}[E1E2][E1]=\frac{1}{k^2}[E1]^2[E2] \quad (S5)$$

$$[(E1)_3E2]=\frac{1}{k}[(E1)_2E2][E1]=\frac{1}{k^3}[E1]^3[E2], \quad (S6)$$

where k is the interaction equilibrium constant between 4CL3 and 4CL5, which is defined as : dissociation constant (k_d) / association constant (k_a) of two enzymes.

The binding between enzyme-complexes and substrates yields

$$[(E1)_3E2S]=\frac{1}{K_{m_2}}[(E1)_3E2][S]=\frac{1}{k^3\cdot K_{m_2}}[E1]^3[E2][S] \quad (S7)$$

$$[(E1)_3E2(S)_2]^*=\frac{1}{K_{is}}[(E1)_3E2S][S]=\frac{1}{k^3\cdot K_{m_2}\cdot K_{is}}[E1]^3[E2][S]^2 \quad (S8)$$

- Conservation of Total Enzyme

Dimers and trimers were not included in the derivation of equations (S9) and (S10) because they were undetected for both recombinant proteins and in SDX when both 4CL3 and 4CL5 are present. This model was built under the simplifying assumption that dimer and trimer formation are transient in the reversible reaction.

The total (t) 4CL3 enzyme concentration is equal to the sum of species with 4CL3:

$$[E1t]=[E1]+[E1S]+3\cdot[(E1)_3E2]+3\cdot[(E1)_3E2S]+3\cdot\left[(E1)_3E2(S)_2\right]^* \quad (S9)$$

The total (t) 4CL5 enzyme concentration is equal to the sum of species with 4CL5:

$$[E2t]=[E2]+[E2S]+[E2(S)_2]^*+[(E1)_3E2]+[(E1)_3E2S]+[(E1)_3E2(S)_2]^* \quad (S10)$$

C. Derivation of Rate Equation

- Definition of Unknown Parameters: k_1, k_2, γ

• *k_1 definition*

We assume that each enzyme component, involved in enzyme-enzyme interactions, can be represented by constant proportions of total enzyme concentrations of 4CL3 or 4CL5: [E1] = α * [E1t] , [E2] = β * [E2t], where α and β are constants between 0 and 1. These proportions are constant under the simplifying assumption that other components, such as substrates, and enzyme-enzyme intermediates do not significantly impact the proportions of 4CL3 and 4CL5 in the tetrameric complex.

These conditions yield

$$\frac{1}{k^3}[E1]^2[E2]=\frac{\alpha^2\beta}{k^3}[E1t]^2[E2t]=\frac{1}{(k_1)^3}[E1t]^2[E2t], \qquad 0<\alpha^2\beta<1 \quad (S11)$$

where k is the unknown equilibrium constant between 4CL3 and 4CL5, α is the proportion of 4CL3 in each enzyme-enzyme binding in terms of the total 4CL3 enzyme concentration, and β is the proportion of 4CL5 in each enzyme-enzyme binding in terms of the total 4CL5 enzyme concentration. k_1 is a new constant involving the effects of α, β, and k on the rate equation. It is defined as:

$$\frac{1}{(k_1)^3}=\frac{\alpha^2\beta}{k^3} \quad (S12)$$

• *k_2 definition*

The enzyme concentration of 4CL3 involved in each enzyme-enzyme binding can be represented as the constant proportion of total enzyme concentrations of 4CL3: [E1] = α * [E1t], based on the conditions mentioned as above in k_1 definition.

These conditions yield

$$\frac{1}{k^3}[E1]^3=\frac{\alpha}{k^3}[E1t]^3=\frac{1}{(k_2)^3}[E1t]^2, \qquad 0<\alpha^3<1 \quad (S13)$$

where the definitions of k and α are the same as the definitions of those constants in the k_1 definition. k_2 is a new constant involving the effects of k and α on the rate equation. It is defined as:

$$\frac{1}{(k_2)^3}=\frac{\alpha^3}{k^3} \quad \text{(S14)}$$

- γ *definition*

γ represents the reaction rate constant for the enzyme-complex.

- [E1] Derivation

Equation S1, S2, S7, and S8 are substituted in S9, which is then written as:

$$[E1t]=[E1]+\frac{1}{K_{m_1}}[E1][S]+\frac{3}{k^3}[E1]^3[E2]+\frac{3}{k^3\cdot K_{m_2}}[E1]^3[E2][S]+\frac{3}{k^3\cdot K_{m_2}\cdot K_{is}}[E1]^3[E2][S]^{2^*} \quad \text{(S15)}$$

This yields

$$[E1]=\frac{[E1t]}{\left(1+\frac{[S]}{K_{m_1}}\right)+\frac{3}{k^3}[E1]^2[E2]\cdot\left(1+\frac{[S]}{K_{m_2}}+\frac{[S]^2}{K_{m_2}\cdot K_{is}}\right)^*} \quad \text{(S16)}$$

Using Equation S11, The expression of [E1] is written as:

$$[E1]=\frac{[E1t]}{\left(1+\frac{[S]}{K_{m_1}}\right)+\frac{3}{k_1^3}[E1t]^2[E2t]\cdot\left(1+\frac{[S]}{K_{m_2}}+\frac{[S]^2}{K_{m_2}\cdot K_{is}}\right)^*} \quad \text{(S17)}$$

- [E2] Derivation

Equation S2, S3, S6, S7, and S8 are substituted in Equation S10, which is then written as:

$$[E2t]=[E2]+\frac{1}{K_{m_2}}[E2][S]+\frac{1}{K_{m_2}\cdot K_{is}}[E2][S]^{2^{*}}+\frac{1}{k^3}[E1]^3[E2]+\frac{1}{k^3\cdot K_{m_2}}[E1]^3[E2][S]$$

$$+\frac{1}{k^3\cdot K_{m_2}\cdot K_{is}}[E1]^3[E2][S]^{2^{*}} \quad (S18)$$

This yields

$$[E2]=\frac{[E2t]}{(1+\frac{[S]}{K_{m_2}}+\frac{[S]^{2^{*}}}{K_{m_2}\cdot K_{is}})\cdot(1+\left(\frac{[E1]}{k}\right)^3)} \quad (S19)$$

Using Equation S13, The expression of [E2] is written as:

$$[E2]=\frac{[E2t]}{(1+\frac{[S]}{K_{m_2}}+\frac{[S]^{2^{*}}}{K_{m_2}\cdot K_{is}})\cdot(1+\left(\frac{[E1t]}{k_2}\right)^3)} \quad (S20)$$

- Rate Equation

In our models, the product is formed by three enzymatic reactions: 1) free 4CL3, 2) free 4CL5, and 3) the 4CL3-4CL5 complex. Dimer and trimer formation are transient in the reversible reaction and do not contribute significantly to product formation. The total rate equation is represented by the sum of the reactions of enzyme-substrate by free 4CL3, free 4CL5, and the reaction of enzyme-complex- substrate by 4CL3-4CL5, which is written as:

$$v = k_{cat_1}[E1S] + k_{cat_2}[E2S] + \gamma\cdot k_{cat_2}[(E1)_3E2S]. \quad (S21)$$

Equation S21 can be changed using Equation S1, S2, and S7 to:

$$v = \frac{k_{cat_1}}{K_{m_1}}[E1][S] + \frac{k_{cat_2}}{K_{m_2}}[E2][S] + \frac{\gamma \cdot k_{cat_2}}{k^3 \cdot K_{m_2}}[E1]^3[E2][S] \quad \text{(S22)}$$

Considering the derived expressions of [E1] and [E2] and after some algebraic manipulation (See Equation S17 and S20 above), the final rate equation is

$$v = \frac{k_{cat_1}[E1t][S]}{K_{m_1} + [S] + \frac{3 \cdot K_{m_1}[E1t]^2[E2t]}{k_1^3}\left(1 + \frac{[S]}{K_{m_2}} + \frac{[S]^2}{K_{m_2} \cdot K_{is}}^*\right)} + \frac{k_{cat_2}[E2t][S]\left(1 + \gamma \cdot \left(\frac{[E1t]}{k_2}\right)^3\right)}{\left(K_{m_2} + [S] + \frac{[S]^2}{K_{is}}^*\right) \cdot \left(1 + \left(\frac{[E1t]}{k_2}\right)^3\right)} \quad \text{(S23)}$$

II. Enzymatic Reaction Equation of the Interaction of 4CL3 and 4CL5 with Multiple Substrate Inhibition

A. Diagrams

Figure 8A (4-coumaric acid), Supplemental Figure 4A online (caffeic acid)

B. Assumptions

The enzymatic reactions involved in the interaction of 4CL3 and 4CL5 with multiple substrates also follow the general Michaelis-Menten kinetic assumptions as the process in Section I. Terms, with *, represent the self-inhibition effect in each equation, and are applied in the model with caffeic acid as a primary substrate only.

- Quasi-equilibrium

The association and disassociation between the components, related to enzymes, substrates, and inhibitors, are in binding equilibrium.

The equations representing enzyme-substrate or enzyme-enzyme complexes are the same as the expressions from Equation S1 to Equation S8 in Section I.

The competitive inhibitions of 4CL3 are represented by

$$[E1I1] = \frac{1}{K3_{ic_1}}[E1][I1] \quad \text{(S24)}$$

$$[\mathrm{E1I2}]=\frac{1}{K3_{\mathrm{ic}_2}}[\mathrm{E1}][\mathrm{I2}] \quad \text{(S25)}$$

where $K3_{\mathrm{ic}_1}$ and $K3_{\mathrm{ic}_2}$ are the competitive inhibition constants for 4CL3.

The competitive and uncompetitive inhibitions of 4CL5 are represented by

$$[\mathrm{E2I1}]=\frac{1}{K5_{\mathrm{ic}_1}}[\mathrm{E2}][\mathrm{I1}] \quad \text{(S26)}$$

$$[\mathrm{E2I2}]=\frac{1}{K5_{\mathrm{ic}_2}}[\mathrm{E2}][\mathrm{I2}] \quad \text{(S27)}$$

$$[\mathrm{E2SI1}]=\frac{1}{K5_{5\mathrm{iu}_1}}[\mathrm{E2S}][\mathrm{I1}]=\frac{1}{K_{\mathrm{m}_2}\cdot K5_{\mathrm{iu}_1}}[\mathrm{E2}][\mathrm{S}][\mathrm{I1}] \quad \text{(S28)}$$

$$[\mathrm{E2SI2}]=\frac{1}{K5_{\mathrm{iu}_2}}[\mathrm{E2S}][\mathrm{I2}]=\frac{1}{K_{\mathrm{m}_2}\cdot K5_{\mathrm{iu}_2}}[\mathrm{E2}][\mathrm{S}][\mathrm{I2}] \quad \text{(S29)}$$

where $K5_{\mathrm{ic}_1}$ and $K5_{\mathrm{ic}_2}$ are the competitive inhibition constants for 4CL5 and $K5_{\mathrm{iu}_1}$ and $K5_{\mathrm{iu}_2}$ are the uncompetitive inhibition constants for 4CL5.

The competitive and uncompetitive inhibitions of the enzyme-complex are represented by

$$[(\mathrm{E1})_3\mathrm{E2I1}]=\frac{1}{K5_{\mathrm{ic}_1}}[(\mathrm{E1})_3\mathrm{E2}][\mathrm{I1}]=\frac{1}{k^3\cdot K5_{\mathrm{ic}_1}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{I1}] \quad \text{(S30)}$$

$$[(\mathrm{E1})_3\mathrm{E2I2}]=\frac{1}{K5_{\mathrm{ic}_2}}[(\mathrm{E1})_3\mathrm{E2}][\mathrm{I2}]=\frac{1}{k^3\cdot K5_{\mathrm{ic}_2}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{I2}] \quad \text{(S31)}$$

$$[(\mathrm{E1})_3\mathrm{E2SI1}]=\frac{1}{K5_{\mathrm{iu}_1}}[(\mathrm{E1})_3\mathrm{E2S}][\mathrm{I1}]=\frac{1}{k^3\cdot K_{\mathrm{m}_2}\cdot K5_{\mathrm{iu}_1}}[\mathrm{E1}]^3[\mathrm{E2}][S][\mathrm{I1}] \quad \text{(S32)}$$

$$[(E1)_3E2SI2]=\frac{1}{K5_{iu_2}}[(E1)_3E2S][I1]=\frac{1}{k^3\cdot K_{m_2}\cdot K5_{iu_2}}[E1]^3[E2][S][I2] \quad (S33)$$

where we assume that the inhibition constants for the enzyme-complex use the same values as the inhibition constants for 4CL5.

- Conservation of Total Enzyme

The total (t) 4CL3 enzyme concentration is equal to the sum of species with 4CL3:

$$[E1t]=[E1]+[E1S]+[E1I1]+[E1I2]+3\cdot[(EI)_3E2]+3\cdot[(EI)_3E2S]+3\cdot[(EI)_3E2I1]+3\cdot[(EI)_3E2I2]$$
$$+3\cdot[(EI)_3E2SI1]+3\cdot[(EI)_3E2SI2]+3\cdot[(EI)_3E2(S)_2]^* \quad (S34)$$

The total (t) 4CL5 enzyme concentration is equal to the sum of species with 4CL5:

$$[E2t]=[E2]+[E2S]+[E2I1]+[E2I2]+[E2(S)_2]^*+[E2SI1]+[E2SI2]+[(EI)_3E2]+[(EI)_3E2S]$$
$$+[(EI)_3E2I1]+[(EI)_3E2I2]+[(EI)_3E2SI1]+[(EI)_3E2SI2]+[(EI)_3E2(S)_2]^* \quad (S35)$$

C. Derivation of Rate Equation

- Definition of Unknown Parameters: k_1, k_2, γ

The same definition is applied as that in Section I.

- [E1] Derivation

Equation S1, S6, S7, S8, S24, S25, S30, S31, S32, and S33 are substituted in Equation S34, which is written as:

$$[E1t]=[E1]+\frac{1}{K_{m_1}}[E1][S]+\frac{1}{K3_{ic_1}}[E1][I1]+\frac{1}{K3_{ic_2}}[E1][I2]+\frac{3}{k^3}[E1]^3[E2]+\frac{3}{k^3\cdot K_{m_2}}[E1]^3[E2][S]$$

$$+\frac{3}{k^3\cdot K5_{\mathrm{ic}_1}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{I1}]+\frac{3}{k^3\cdot K5_{\mathrm{ic}_2}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{I2}]+\frac{3}{k^3\cdot K_{\mathrm{m}_2}\cdot K5_{\mathrm{ic}_1}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{S}][\mathrm{I1}]$$

$$+\frac{3}{k^3\cdot K_{\mathrm{m}_2}\cdot K5_{\mathrm{iu}_2}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{S}][\mathrm{I2}]+\frac{3}{k^3\cdot K_{\mathrm{m}_2}\cdot K_{\mathrm{is}}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{S}]^{2^*} \quad \text{(S36)}$$

This yields

$$[\mathrm{E1}]=\frac{[\mathrm{E1t}]}{\left(1+\frac{[\mathrm{S}]}{K_{\mathrm{m}_1}}+\frac{[\mathrm{I1}]}{K3_{\mathrm{ic}_1}}+\frac{[\mathrm{I2}]}{K3_{\mathrm{ic}_2}}\right)+\frac{3}{k^3}[\mathrm{E1}]^2[\mathrm{E2}]\cdot\left(1+\frac{[\mathrm{I1}]}{K5_{\mathrm{ic}_1}}+\frac{[\mathrm{I2}]}{K5_{\mathrm{ic}_2}}+\frac{[\mathrm{S}]}{K_{\mathrm{m}_1}}\cdot\left(1+\frac{[\mathrm{I1}]}{K5_{\mathrm{iu}_1}}+\frac{[\mathrm{I2}]}{K5_{\mathrm{iu}_2}}+\frac{[\mathrm{S}]^*}{K_{\mathrm{is}}}\right)\right)} \quad \text{(S37)}$$

Using Equation S11, The expression of [E1] is written as:

$$[\mathrm{E1}]=\frac{[\mathrm{E1t}]}{\left(1+\frac{[\mathrm{S}]}{K_{\mathrm{m}_1}}+\frac{[\mathrm{I1}]}{K3_{\mathrm{ic}_1}}+\frac{[\mathrm{I2}]}{K3_{\mathrm{ic}_2}}\right)+\frac{3}{{k_1}^3}[\mathrm{E1t}]^2[\mathrm{E2t}]\cdot\left(1+\frac{[\mathrm{I1}]}{K5_{\mathrm{ic}_1}}+\frac{[\mathrm{I2}]}{K5_{\mathrm{ic}_2}}+\frac{[\mathrm{S}]}{K_{\mathrm{m}_1}}\cdot\left(1+\frac{[\mathrm{I1}]}{K5_{\mathrm{iu}_1}}+\frac{[\mathrm{I2}]}{K5_{\mathrm{iu}_2}}+\frac{[\mathrm{S}]^*}{K_{\mathrm{is}}}\right)\right)} \quad \text{(S38)}$$

- [E2] Derivation

Equation S2, S3, S6, S7, S8, S26, S27, S28, S29, S30, S31, S32, and S33 are substituted in Equation S35, which is written as:

$$[\mathrm{E2t}]=[\mathrm{E2}]+\frac{1}{K_{\mathrm{m}_2}}[\mathrm{E2}][\mathrm{S}]+\frac{1}{K5_{\mathrm{ic}_1}}[\mathrm{E2}][\mathrm{I1}]+\frac{1}{K5_{\mathrm{ic}_2}}[\mathrm{E2}][\mathrm{I2}]+\frac{1}{K_{\mathrm{m}_2}\cdot K_{\mathrm{is}}}[\mathrm{E2}][\mathrm{S}]^{2^*}+\frac{1}{K_{\mathrm{m}_2}\cdot K_{5\mathrm{iu}_1}}[\mathrm{E2}][\mathrm{S}][\mathrm{I1}]$$

$$+\frac{1}{K_{\mathrm{m}_2}\cdot K5_{\mathrm{iu}_2}}[\mathrm{E2}][\mathrm{S}][\mathrm{I2}]+\frac{1}{k^3}[\mathrm{E1}]^3[\mathrm{E2}]+\frac{1}{k^3\cdot K_{\mathrm{m}_2}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{S}]+\frac{1}{k^3\cdot K5_{\mathrm{ic}_1}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{I1}]$$

$$+\frac{1}{k^3\cdot K5_{\mathrm{ic}_2}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{I2}]+\frac{1}{k^3\cdot K_{\mathrm{m}_2}\cdot K5_{\mathrm{iu}_1}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{S}][\mathrm{I1}]+\frac{1}{k^3\cdot K_{\mathrm{m}_2}\cdot K5_{\mathrm{iu}_2}}[\mathrm{E1}]^3[\mathrm{E2}][\mathrm{S}][\mathrm{I2}]$$

$$+\frac{1}{k^3 \cdot K_{m_2} \cdot K_{is}}[E1]^3[E2][S]^2 \quad ^* \tag{S39}$$

This yields

$$[E2]=\frac{[E2t]}{(1+\frac{[I1]}{K5_{ic_1}}+\frac{[I2]}{K5_{ic_2}}+\frac{[S]}{K_{m_2}}\cdot\left(1+\frac{[I1]}{K5_{iu_1}}+\frac{[I2]}{K5_{iu_2}}+\frac{[S]}{K_{is}}^*\right))\cdot(1+\left(\frac{[E1]}{k}\right)^3)} \tag{S40}$$

Using Equation S13, The expression of [E2] is written as:

$$[E2]=\frac{[E2t]}{(1+\frac{[I1]}{K5_{ic_1}}+\frac{[I2]}{K5_{ic_2}}+\frac{[S]}{K_{m_2}}\cdot\left(1+\frac{[I1]}{K5_{iu_1}}+\frac{[I2]}{K5_{iu_2}}+\frac{[S]}{K_{is}}^*\right))\cdot(1+\left(\frac{[E1t]}{k_2}\right)^3)} \tag{S41}$$

- Rate Equation

The total rate equation is written as:

$$v = k_{cat_1}[E1S] + k_{cat_2}[E2S] + \gamma \cdot k_{cat_2}[(E1)_3E2S] \tag{S42}$$

Equation S42 can be changed using Equation S1, S2, and S7:

$$v = \frac{k_{cat_1}}{K_{m_1}}[E1][S] + \frac{k_{cat_2}}{K_{m_2}}[E2][S] + \frac{\gamma \cdot k_{cat_2}}{k^3 \cdot K_{m_2}}[E1]^3[E2][S] \tag{S43}$$

Considering the derived expressions of [E1] and [E2] above, the final rate equation is

$$v=\frac{k_{cat_1}[E1t][S]}{K_{m_1}\cdot(1+\frac{[I1]}{K3_{ic_1}}+\frac{[I2]}{K3_{ic_2}})+[S]+\frac{3\cdot K_{m_1}[E1t]^2[E2t]}{{k_1}^3}\cdot(1+\frac{[I1]}{K5_{ic_1}}+\frac{[I2]}{K5_{ic_2}}+\frac{[S]}{K_{m_2}}\cdot(1+\frac{[I1]}{K5_{iu_1}}+\frac{[I2]}{K5_{iu_2}}+\frac{[S]^*}{K_{is}}))}$$

$$+\frac{k_{cat_2}[E2t][S](1+\gamma\cdot(\frac{[E1t]}{k_2})^3)}{(K_{m_2}\cdot(1+\frac{[I1]}{K5_{ic_1}}+\frac{[I2]}{K5_{ic_2}})+[S]\cdot(1+\frac{[I1]}{K5_{iu_1}}+\frac{[I2]}{K5_{iu_2}}+\frac{[S]^*}{K_{is}}))\cdot(1+(\frac{[E1t]}{k_2})^3)} \quad \text{(S44)}$$

REFERENCES

Klipp, E. (2005). Systems biology in practice: concepts, implementation, and application. Weinheim: Wiley-VCH, pp 144-153.

Chou, T.C., and Talaly, P. (1977). A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems. J. Biol. Chem. **252:** 6438–6442.