Supplementary Material

A Basic Enzyme Reaction

In this section we show the additional results, concerning the basic enzyme reaction, that were discussed in the main paper. First we discuss the fixed points of the basic enzyme reaction. Then we show that the systems converges to its fixed point whenever it exists. Finally, we derive a simple marginal model from the CCM representation of the basic enzyme reaction.

A.1 Fixed points

The fixed points of the basic enzyme reaction, for all intervened systems, are given in Table 2. For any intervention, these are obtained by solving the system of equations that one gets by considering the causal constraints in the CCM in (16) to (25) that are active under that specific intervention. That is, we take all equations for which the intervention is in the activation set.

Table 2: Fixed points of the basic enzyme reaction, where $y = \frac{1}{2}\sqrt{(e_0 - s_0)^2 + 4\frac{k_0(k_{-1}+k_2)}{k_1 k_2}}$.

intervention	S	C	E	P
none	$\frac{k_0+k_{-1}\frac{k_0}{k_2}}{k_1(e_0+c_0-\frac{k_0}{k_2})}$	$\frac{k_0}{k_2}$	$e_0 + c_0 - \frac{k_0}{k_2}$	$\frac{k_0}{k_3}$
do($S = s$)	s	$\frac{k_1 s(e_0+c_0)}{k_{-1}+k_2+k_1 s}$	$\frac{(k_{-1}+k_2)(e_0+c_0)}{k_{-1}+k_2+k_1 s}$	$\frac{k_2}{k_3}\frac{k_1 s(e_0+c_0)}{k_{-1}+k_2+k_1 s}$
do($C = c$), $c = \frac{k_0}{k_2}$	$\frac{(s_0-e_0)}{2} + y$	c	$\frac{-(s_0-e_0)}{2} + y$	$\frac{k_2}{k_3}c$
do($C = c$), $c \neq \frac{k_0}{k_2}$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$
do($E = e$)	$\frac{k_0+k_{-1}\frac{k_0}{k_2}}{k_1 e}$	$\frac{k_0}{k_2}$	e	$\frac{k_0}{k_3}$
do($P = p$)	$\frac{k_0+k_{-1}\frac{k_0}{k_2}}{k_1(e_0+c_0-\frac{k_0}{k_2})}$	$\frac{k_0}{k_2}$	$e_0 + c_0 - \frac{k_0}{k_2}$	p
do($S = s, C = c$)	s	c	$\frac{k_{-1}+k_2}{k_1}\frac{c}{s}$	$\frac{k_2}{k_3}c$
do($S = s, E = e$)	s	$\frac{k_1}{k_{-1}+k_2}se$	e	$\frac{k_2}{k_3}\frac{k_1}{k_{-1}+k_2}se$
do($S = s, P = p$)	s	$\frac{k_1 s(e_0+c_0)}{k_{-1}+k_2+k_1 s}$	$\frac{(k_{-1}+k_2)(e_0+c_0)}{k_{-1}+k_2+k_1 s}$	p
do($C = c, E = e$)	$\frac{k_0+k_{-1}c}{k_1 e}$	c	e	$\frac{k_2}{k_3}c$
do($C = c, P = p$), $c = \frac{k_0}{k_2}$	$\frac{(s_0-e_0)}{2} + y$	c	$\frac{-(s_0-e_0)}{2} + y$	p
do($C = c, P = p$), $c \neq \frac{k_0}{k_2}$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$
do($E = e, P = p$)	$\frac{k_0+k_{-1}\frac{k_0}{k_2}}{k_1 e}$	$\frac{k_0}{k_2}$	e	p
do($S = s, C = c, E = e$)	s	c	e	$\frac{k_2}{k_3}c$
do($S = s, C = c, P = p$)	s	c	$\frac{k_{-1}+k_2}{k_1}\frac{c}{s}$	p
do($S = s, E = e, P = p$)	s	$\frac{k_1}{k_{-1}+k_2}se$	e	p
do($C = c, E = e, P = p$)	$\frac{k_0+k_{-1}c}{k_1 e}$	c	e	p
do($S = s, C = c, E = e, P = p$)	s	c	e	p

A.2 Convergence results for the basic enzyme reaction

In this section, we show that the basic enzyme reaction always converges to its fixed point, as long as it exists. We also show that the intervened basic enzyme reaction has the same property. To prove this result we rely on both explicit calculations and a convergence property of so-called cooperative systems that we obtained from Belgacem and Gouzé (2012). To prove convergence for the observed system and the system after interventions on P and E, we use the latter technique. Convergence to the equilibrium solution after interventions on S and C can be shown by explicit calculation. The convergence results for combinations of interventions can be obtained by a trivial extension of the arguments that were used in the other cases.

A.2.1 Cooperativity in the basic enzyme reaction

To show that the basic enzyme reaction converges to a unique equilibrium, if it exists, we first state a result that we obtained from Belgacem and Gouzé (2012): cooperative systems as in Definition 10 have the attractive convergence property in Proposition 2.

Definition 10. A system of ODEs $\dot{\boldsymbol{X}}$ is *cooperative* if the Jacobian matrix has non-negative off-diagonal elements, or there exists an integer k such that the Jabobian has $(k \times k)$ and $(n-k) \times (n-k)$ main diagonal matrices with nonnegative off-diagonal entries and the rectangular off-diagonal submatrices have non-positive entries.

Proposition 2. Let $\dot{\boldsymbol{X}} = \boldsymbol{f}(\boldsymbol{X})$ be a cooperative system with a fixed point $\boldsymbol{x}^*$. If there exist two points $\boldsymbol{x}_{\text{min}}, \boldsymbol{x}_{\text{max}} \in \boldsymbol{\mathcal{X}}$ such that $\boldsymbol{x}_{\text{min}} \leq \boldsymbol{x}^* \leq \boldsymbol{x}_{\text{max}}$ and $\boldsymbol{f}(\boldsymbol{x}_{\text{min}}) \geq 0$ and $\boldsymbol{f}(\boldsymbol{x}_{\text{max}}) \leq 0$, then the hyperrectangle betweeen $\boldsymbol{x}_{\text{min}}$ and $\boldsymbol{x}_{\text{max}}$ is invariant[9] and for almost all initial conditions inside this rectangle the solution converges to $\boldsymbol{x}^*$.

A.2.2 Convergence of the observed system

Recall that the dynamics of the basic enzyme reaction are given by

$$\dot{S}(t) = k_0 - k_1 S(t)E(t) + k_{-1}C(t), \tag{29}$$
$$\dot{E}(t) = -k_1 S(t)E(t) + (k_{-1} + k_2)C(t), \tag{30}$$
$$\dot{C}(t) = k_1 S(t)E(t) - (k_{-1} + k_2)C(t), \tag{31}$$
$$\dot{P}(t) = k_2 C(t) - k_3 P(t), \tag{32}$$
$$S(0) = s_0, \quad E(0) = e_0, \quad C(0) = c_0, \quad P(0) = p_0, \tag{33}$$

where $\boldsymbol{x}_0 = (s_0, e_0, c_0, p_0)$ are the *initial conditions* of the system.

The analysis in Belgacem and Gouzé (2012) of the basic enzyme reaction makes use of Proposition 2, but also includes feedback from P to C. In this section, we repeat their analysis on our sligthly different model. Note that the arguments given in this section can also be applied to the system where P is intervened upon.

We start by rewriting the system of ODEs in equation (29) to (32), by using the fact that $\dot{E}(t) + \dot{C}(t) = 0$ so that $E(t) = e_0 + c_0 - C(t)$:

$$\dot{S}(t) = k_0 - k_1 S(t)(e_0 + c_0 - C(t)) + k_{-1}C(t), \tag{34}$$
$$\dot{C}(t) = k_1 S(t)(e_0 + c_0 - C(t)) - (k_{-1} + k_2)C(t), \tag{35}$$
$$\dot{P}(t) = k_2 C(t) - k_3 P(t). \tag{36}$$

Cooperativity The corresponding Jacobian matrix is given by,

$$J(S, C, P) = \begin{pmatrix} -k_1(e_0 + c_0 - C(t)) & k_{-1} + k_1 S(t) & 0 \\ k_1(e_0 + c_0 - C(t)) & -(k_{-1} + k_2) - k_1 S(t) & 0 \\ 0 & k_2 & -k_3 \end{pmatrix}. \tag{37}$$

Since all off-diagonal elements in the Jacobian matrix are nonnegative, the observational system is a cooperative system by Definition 10.

Convergence From Table 2 we find that the observed system has a unique (positive) fixed point as long as $e_0 + c_0 > \frac{k_0}{k_2}$. We want to use Proposition 2 to show that the system converges to this fixed point, so we need to find $\boldsymbol{x}_{\text{min}}$ and $\boldsymbol{x}_{\text{max}}$ so that all three derivatives are nonnegative and nonpositive respectively.

For $\boldsymbol{x}_{\text{min}} = (0, 0, 0)$, then $\dot{S} = k_0 > 0$ and $\dot{C} = \dot{P} = 0$ so all derivatives are nonnegative. The upper vertex must be

[9] An invariant set is a set with the property that once a trajectory of a dynamical set enters it, it cannot leave.

chosen so that all derivative are non-positive:

$$
\begin{aligned}
\dot{S} \leq 0 &\iff S \geq \frac{k_0 + k_{-1}C}{k_1(e_0 + c_0 - C)}, \\
\dot{C} \leq 0 &\iff S \geq \frac{(k_{-1} + k_2)C}{k_1(e_0 + c_0 - C)}, \\
\dot{P} \leq 0 &\iff P \geq \frac{k_2}{k_3}C.
\end{aligned}
$$

The basic enzyme reaction only has a fixed point as long as $C < e_0 + c_0$ (otherwise $\dot{S}(t) > 0$). If we let C approach $e_0 + c_0$, then the inequality constraints on the derivatives are satisfied as S and P go to infinity. More formally we can choose

$$
\boldsymbol{x}_{\text{max}} = (S = \max\left(\frac{k_0 + k_{-1}C}{k_1(e_0 + c_0 - C)}, \frac{(k_{-1} + k_2)C}{k_1(e_0 + c_0 - C)}\right), C = e_0 + c_0 - \epsilon, P = \frac{k_2}{k_3}C + \frac{1}{\epsilon}).
$$

When ϵ approaches zero, both S and P go to infinity and all derivatives are nonpositive. Hence, by Proposition 2, the system converges to its fixed point for almost all valid initial values of S, C, and P (for which the fixed point exists).

A.2.3 Intervention on E

Similarly, we can also show that the system where E is targeted by an intervention that sets it equal to e, converges to the (unique) equilibrium in Table 2. The intervened system of ODEs is given by

$$
\begin{aligned}
\dot{S} &= k_0 - k_1 e S + k_{-1}C, \\
\dot{C} &= k_1 e S - (k_{-1} + k_2)C, \\
\dot{P} &= k_2 C - k_3 P.
\end{aligned}
$$

The Jacobian is given by

$$
J(S, C, P) = \begin{pmatrix} -k_1 e & k_{-1} & 0 \\ k_1 e & -(k_{-1} + k_2) & 0 \\ 0 & k_2 & -k_3 \end{pmatrix}. \tag{38}
$$

Since all off-diagonal elements are nonnegative this is a cooperative system by Definition 10.

All derivatives are nonnegative at the point $(S, C, P) = (0, 0, 0)$, and all derivatives are nonpositive at the point (s, c, p) where

$$
\begin{aligned}
s &= \max\left(\frac{k_{-1}c + k_0}{k_1 e}, \frac{(k_{-1} + k_2)c}{k_1 e}\right), \\
p &= \frac{k_2}{k_3}c,
\end{aligned}
$$

where $c \to \infty$. We then apply Proposition 2 to show that the intervened system converges to the equilibrium value from all valid initial values.

A.2.4 Intervention on S

We show that the system converges to the equilibrium solution after an intervention on S by explicit calculation. The intervened system of ODEs is given by

$$
\begin{aligned}
\dot{S}(t) &= 0, \\
\dot{E}(t) &= -k_1 s E(t) + (k_{-1} + k_2)C(t), \\
\dot{C}(t) &= k_1 s E(t) - (k_{-1} + k_2)C(t), \\
\dot{P}(t) &= k_2 C(t) - k_3 P(t).
\end{aligned}
$$

Since $\dot{C}(t) + \dot{E}(t) = 0$, we can write $E(t) = e_0 + c_0 - C(t)$, resulting in the following differential equation

$$\begin{aligned} \dot{C}(t) &= k_1 s(e_0 + c_0 - C(t)) - (k_{-1} + k_2)C(t), && (39) \\ &= -(k_1 s + k_{-1} + k_2)C(t) + k_1 s(e_0 + c_0). && (40) \end{aligned}$$

We take the limit $t \to \infty$ of the solution to the initial value problem to obtain

$$C^* = \lim_{t\to\infty} \frac{k_1 s(e_0 + c_0)}{(k_1 s + k_1 + k_2)} + e^{-(k_1 s + k_{-1} + k_2)t} = \frac{k_1 s(e_0 + c_0)}{(k_1 s + k_{-1} + k_2)}. \quad (41)$$

The result for E follows from the fact that $E(t) = e_0 + c_0 - C(t)$. The result for P follows by explicitly solving the differential equation and taking the limit $t \to \infty$.

A.2.5 Intervention on C

There is no equilibrium solution when the intervention targeting C does not have value $\frac{k_0}{k_2}$, as can be seen from Table 2. To show that the system converges when the equilibrium solution exists, we can explicitly solve the initial value problem and take the limit $t \to \infty$. The intervened system of ODEs after an intervention do$(C = \frac{k_0}{k_2})$ is given by

$$\begin{aligned} \dot{S}(t) &= -k_1 S(t)E(t) + (k_{-1} + k_2)\frac{k_0}{k_2} = -k_1 S(t)E(t) + k, \\ \dot{E}(t) &= -k_1 S(t)E(t) + (k_{-1} + k_2)\frac{k_0}{k_2} = -k_1 S(t)E(t) + k, \\ \dot{C}(t) &= 0, \\ \dot{P}(t) &= k_0 - k_3 P(t), \end{aligned}$$

where we set $k = (k_{-1} + k_2)\frac{k_0}{k_2}$ for brevity.

The initival value problem for P can be solved explicitly, and by taking the limit $t \to \infty$ we obtain

$$P^* = \lim_{t\to\infty} P(t) = \lim_{t\to\infty} \frac{k_0}{k_3} + c \cdot e^{-k_3 t} = \frac{k_0}{k_3},$$

which is the same as the equilibrium solution in Table 2.

The solution for S is more involved. First we substitute $E(t) = S(t) - (s_0 - e_0)$ (since $\dot{S}(t) - \dot{E}(t) = 0$) which gives us the following differential equation

$$\dot{S}(t) = -k_1 S(t)(S(t) - (s_0 - e_0)) + k = -k_1 S(t)^2 + (s_0 - e_0)k_1 S(t) + k.$$

To solve this differential equation we first divide both sides by $(-k_1(S(t))^2 + (s_0 - e_0)k_1 S(t) + k)$, and integrate both sides with respect to t,

$$\begin{aligned} \int \frac{dS(t)/dt}{-k_1 S(t)^2 + (s_0 - e_0)k_1 S(t) + k} dt &= \int 1 dt && (42) \\ \int \frac{dS(t)}{-k_1 S(t)^2 + (s_0 - e_0)k_1 S(t) + k} &= (t + c) && (43) \end{aligned}$$

To evaluate the left-hand side of this equation we want to apply the following standard integral:

$$\int \frac{1}{ax^2 + bx + c} dx = \begin{cases} -\frac{2}{\sqrt{b^2 - 4ac}} \tanh^{-1}\left(\frac{2ax + b}{\sqrt{b^2 - 4ac}}\right) + C, & \text{if } |2ax + b| < \sqrt{b^2 - 4ac}, \\ -\frac{2}{\sqrt{b^2 - 4ac}} \coth^{-1}\left(\frac{2ax + b}{\sqrt{b^2 - 4ac}}\right) + C, & \text{else.} \end{cases} \quad (44)$$

for $b^2 - 4ac > 0$. We first check the condition:

$$b^2 - 4ac = (s_0 - e_0)^2 k_1^2 + 4k_1 k > 0.$$

We now take the first solution to the standard integral (the second solution gives the same limiting result for S, as we will see later on). We apply the first solution in (44) to (43) to obtain

$$\frac{2\tanh^{-1}\left(\frac{2k_1S(t)-(s_0-e_0)k_1}{\sqrt{4k_1k+(s_0-e_0)^2k_1^2}}\right)}{\sqrt{4k_1k+(s_0-e_0)^2k_1^2}} = t+c \tag{45}$$

$$\tanh^{-1}\left(\frac{2k_1S(t)-(s_0-e_0)k_1}{\sqrt{4k_1k+(s_0-e_0)^2k_1^2}}\right) = \frac{1}{2}(t+c)\sqrt{4k_1k+(s_0-e_0)^2k_1^2} \tag{46}$$

$$\frac{2k_1S(t)-(s_0-e_0)k_1}{\sqrt{4k_1k+(s_0-e_0)^2k_1^2}} = \tanh\left(\frac{1}{2}(t+c)\sqrt{4k_1k+(s_0-e_0)^2k_1^2}\right), \tag{47}$$

Solving (47) for S gives,

$$S(t) = \frac{1}{2k_1}\left(\tanh\left(\frac{1}{2}(t+c)\sqrt{4k_1k+(s_0-e_0)^2k_1^2}\right)\sqrt{4k_1k+(s_0-e_0)^2k_1^2}+k_1(s_0-e_0)\right).$$

By taking the limit $t\to\infty$, plugging in $k=(k_{-1}+k_2)\frac{k_0}{k_2}$, and rewriting we obtain the equilibrium solution in Table 2:

$$\begin{aligned}\lim_{t\to\infty} S(t) &= \frac{k_1(s_0-e_0)+\sqrt{4k_1k+(s_0-e_0)^2k_1^2}}{2k_1}\\ &= \frac{k_1(s_0-e_0)+\sqrt{4k_1(k_{-1}+k_2)\frac{k_0}{k_2}+(s_0-e_0)^2k_1^2}}{2k_1}\\ &= \frac{1}{2}\left((s_0-e_0)+\sqrt{(s_0-e_0)^2+4\frac{k_0(k_{-1}+k_2)}{k_1k_2}}\right).\end{aligned}$$

Note that if we take the second solution to the standard integral in (44), then we would have ended up with the same solution for $S(t)$ with $\tanh$ replaced by $\coth$, but the limit $\lim_{t\to\infty} S(t)$ would still be the same.

The solution for E follows from the fact that $E(t)=S(t)-(s_0-e_0)$. The solutions for all joint interventions were found by combining the arguments that were given for the single interventions.

A.3 Marginal model

In the paper we presented a marginal model for the basic enzyme reaction. Here we show how it can be derived from the causal constraints in the CCM, which are given by

$$\begin{aligned}
k_0+k_{-1}C-k_1SE&=0, && \mathcal{P}(\mathcal{I}\backslash\{S\}), && (48)\\
k_1SE-(k_{-1}+k_2)C&=0, && \mathcal{P}(\mathcal{I}\backslash\{C\}), && (49)\\
-k_1SE+(k_{-1}+k_2)C&=0, && \mathcal{P}(\mathcal{I}\backslash\{E\}), && (50)\\
k_2C-k_3P&=0, && \mathcal{P}(\mathcal{I}\backslash\{P\}), && (51)\\
C+E-(c_0+e_0)&=0, && \mathcal{P}(\mathcal{I}\backslash\{C,E\}), && (52)\\
S-E-(s_0-e_0)&=0, && \{\{C\},\{C,P\}\}. && (53)
\end{aligned}$$

We obtain the marginal model as follows:

1. Reduce the number of variables that can be targeted by an intervention: $\mathcal{I}'=\{S,E,P\}$.
2. Rewrite the causal constraint in (49) to $C=\frac{k_1SE}{k_{-1}+k_2}$. Note that this equation holds under any intervention in $\mathcal{P}(\mathcal{I}')=\mathcal{P}(\mathcal{I}\backslash\{C\})$. Then substitute this expression for C into equation (48) to obtain

$$\frac{k_0+k_{-1}\frac{k_0}{k_2}}{k_1E}-S=0, \qquad \mathcal{P}(\mathcal{I}'\backslash\{S\}),$$

where the activation set of the causal constraint is given by the intersection $\mathcal{P}(\mathcal{I}\backslash\{S\}) \cap \mathcal{P}(\mathcal{I}')$. Then substitute this expresion for C into equation (51) to obtain

$$\frac{k_2}{k_3}\frac{k_1 SE}{k_{-1}+k_2} - P = 0, \qquad \mathcal{P}(\mathcal{I}'\backslash\{P\}),$$

where the activation set of the causal constraint is given by the intersection $\mathcal{P}(\mathcal{I}\backslash\{P\}) \cap \mathcal{P}(\mathcal{I}')$.

3. Rewrite the causal constraint in (52) to $C = e_0 + c_0 - E$ and note that this equation holds under interventions in $\mathcal{P}(\mathcal{I}'\backslash\{E\})$. Then substitute this expression for C into equation (50) to obtain

$$\frac{(k_{-1}+k_2)(c_0+e_0)}{k_{-1}+k_2+k_1 S} - E = 0, \qquad \mathcal{P}(\mathcal{I}'\backslash\{E\}),$$

where the activation set of the causal constraint is given by the intersection $\mathcal{P}(\mathcal{I}\backslash\{C,E\}) \cap \mathcal{P}(\mathcal{I}'\backslash\{E\})$.

This procedure results in the following marginal model

$$\begin{aligned}
\frac{k_0 + k_{-1}\frac{k_0}{k_2}}{k_1 E} - S &= 0, && \mathcal{P}(\mathcal{I}'\backslash\{S\}),\\
\frac{(k_{-1}+k_2)(c_0+e_0)}{k_{-1}+k_2+k_1 S} - E &= 0, && \mathcal{P}(\mathcal{I}'\backslash\{E\}),\\
\frac{k_2}{k_3}\frac{k_1 SE}{k_{-1}+k_2} - P &= 0, && \mathcal{P}(\mathcal{I}'\backslash\{P\}).
\end{aligned}$$

Because we kept track of the interventions under which each equation is active when we substituted C into the equations of other causal constraints, we preserved the causal structure of the model. That is, the marginal CCM model has the same solutions as the original CCM under interventions in $\mathcal{P}(\mathcal{I}')$.