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# Introduction to the concept of *functioning-dependent structures* in living cells

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**Abstract**

The assembly of proteins into larger structures may confer advantages such as increased resistance to hydrolytic enzymes, metabolite channelling, and reduction of the number of proteins or other active molecules required for cell functioning. We propose the term *functioning-dependent structures* (*FDSs*) for those associations of proteins that are created and maintained by their action in accomplishing a function, as reported in many experiments. Here we model the simplest possible cases of two-partner *FDSs* in which the associations either catalyse or inhibit reactions. We show that *FDSs* may display regulatory properties (e.g., a sigmoidal response or a linear kinetic behaviour over a large range of substrate concentrations) even when the individual proteins are enzymes of the Michaelis–Menten type. The possible involvement of more complicated *FDSs* or of *FDS* networks in real living systems is discussed. From the thermodynamic point of view, *FDS* formation and decay are responsible for an extra production of entropy, which may be considered characteristic of living systems. ***To cite this article: M. Thellier et al., C. R. Biologies 327 (2004).***


**Résumé**

**Introduction au concept de *structures dépendant de leur fonctionnement* dans les cellules vivantes.** L'association de protéines en complexes plurimoléculaires peut leur conférer des avantages tels qu'une augmentation de la résistance aux enzymes d'hydrolyse, la canalisation des métabolites et une réduction du nombre de protéines ou autres molécules actives nécessaires au fonctionnement cellulaire. Nous proposons d'appeler *structures dépendant de leur fonctionnement* (*FDS*) les associations de protéines qui sont créées et maintenues par le fait qu'elles sont en train d'accomplir leur fonction. De telles situations ont été décrites à diverses reprises dans la littérature. Ici, nous avons modélisé les cas les plus simples possibles, c'est à dire les *FDS* à deux partenaires, catalytiques ou inhibitrices. Nous montrons que les *FDS* peuvent présenter des propriétés régulatrices (comportements cinétiques sigmoïdes, ou linéaires sur une vaste plage de concentrations), même lorsque les protéines constitutives de ces *FDS* sont de type Michaélien. L'éventuelle intervention de *FDS* à plus de deux partenaires ou même des réseaux de *FDS*

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dans les systèmes vivants réels est discutée. Du point de vue thermodynamique, l'association et la dissociation des *FDS* conduit à une production d'entropie supplémentaire qui peut être considérée comme caractéristique des systèmes vivants. ***Pour citer cet article : M. Thellier et al., C. R. Biologies 327 (2004).***


*Keywords:* enzymes; enzyme kinetics; sigmoidal curves; linear responses; transient protein associations; entropy production

*Mots-clés :* enzymes ; cinétique enzymatique ; sigmoïdicité ; linéarisation ; associations transitoires de protéines ; production d'entropie

## Abbreviation

*FDS*, functioning-dependent structure

## 1. Introduction

Proteins involved in a cooperative cellular task, such as a metabolic or signalling pathway, are not always randomly distributed but may exist in the form of multimolecular complexes (reviewed in Mathews [1]), which have been termed *metabolons* in the case of metabolic pathways [1–3], *transducons* in the case of signal transduction [4], or, more generally, *hyperstructures* (possibly including not only protein subunits, but also other components such as nucleic acids or lipids and implicating equilibrium as well as non-equilibrium molecular associations) [5].

As a particular case of the above, the complexes might assemble only in an activity-dependent manner, that is, proteins do not associate spontaneously but only when they are actually engaged in the process of transport and/or transformation of a substrate or transduction of a signal [1,5–10]. Demonstrative examples of such behaviour are (*i*) the control of several steps of the glycolytic pathway by metabolite-modulated dynamic enzyme associations [6] and (*ii*) the ATP- and pH-dependent association/dissociation of the $V_1$ and $V_0$ domains of the yeast vacuolar $H^+$-ATPases [11]. We propose to term *functioning-dependent structure* (*FDS*) a dynamic assembly that forms and maintains itself by the very fact that it is accomplishing a task and that disassembles when no longer functioning. Some advantages conferred by molecules being in such assemblies as opposed to being free are obvious (increased resistance to hydrolytic enzymes, substrate channelling, reduction of the number of proteins or other active molecules required for cellular processes). *Metabolite-induced metabolons* [10] exemplify such *FDSs*. In this paper we model the particularly simple example of an enzymatic two-partner *FDS* with a view to unravelling the basic kinetic properties of *FDSs* under steady-state conditions. Then we discuss briefly the possibility of developing *FDS* models of increasing complexity in order to represent subcellular structures more realistically.

## 2. The two-enzyme models

Consider a reaction medium containing two different enzymes, $E$ and $F$, with $E$ catalysing the transformation of $S$ to $P$ and $F$ catalysing the transformation of $P$ to $Q$, i.e.

$$S \underset{}{\overset{E}{\rightleftharpoons}} P \overset{F}{\rightleftharpoons} Q \qquad (1)$$

In the conventional case (Fig. 1), the enzymes $E$ and $F$ work independently of each other according to the series of steps characterised by their rate constants. In this case, the intermediate substance $P$ must go from the enzyme molecule $E$, which has released it, to an enzyme molecule $F$ that binds it for the accomplishment of the overall reaction from $S$ to $Q$. Note that in this and the following figures, the reactions are

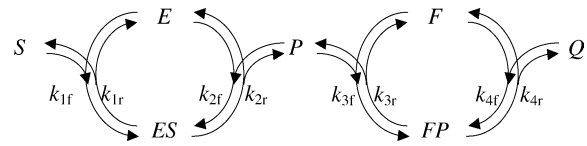


Fig. 1. The conventional case in which two free enzymes, $E$ and $F$, catalyse two sequential reactions (see (1)). The first enzyme, $E$, and the initial substrate, $S$, form a substrate-enzyme complex, $ES$, which releases the product $P$ in the reaction medium, thus regenerating the free enzyme $E$. Then $P$ diffuses at random until it reaches an enzyme $F$ where it is transformed into the final product $Q$, via an enzyme complex $FP$, and which is then released into the reaction medium. The parameters $k_{jf}$ and $k_{jr}$ are the forward and reverse rate constants of each reaction, $j$.

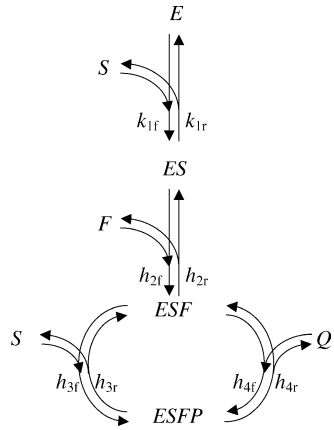


Fig. 2. A 'catalytic' two-enzyme model of *FDS*. In this model, the free enzyme $F$ is not capable of binding and reacting with its substrate, $P$, and the free enzymes, $E$ and $F$, are not capable of assembling with each other. However, the binding of substrate $S$ by enzyme $E$ is responsible for a structural transition that confers on $E$ an ability to bind to enzyme $F$ thus forming the two-partner *functioning-dependent structure ESF*. Then this structure catalyses the transformation of $S$ into $P$ (without releasing $P$ into the reaction medium), the channelling of $P$ to $F$ and the fixation of another $S$ molecule, thus forming the complex *ESFP*. Within this latter complex, $F$ transforms $P$ into $Q$ and releases $Q$ into the reaction medium, thus regenerating *ESF*. In brief, *ESF* catalyses the overall transformation of one $S$ to one $Q$ per cycle. The first step (with the rate constants $k_{1f}$ and $k_{1r}$) is identical to that in the conventional case in which the two enzymes are not assembled into a *FDS* (Fig. 1). The parameters $h_{jf}$ and $h_{jr}$ are the rate constants of each reaction, $j$, involved in the *FDS* formation and functioning.

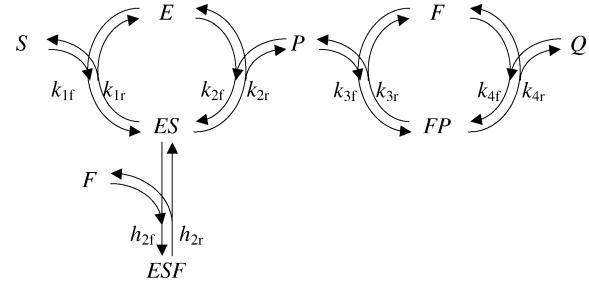


Fig. 3. An 'inhibitory' two-enzyme model of *FDS*. In this case, some of the enzymes, $E$ and $F$, are sequestered into a *FDS* in which they are inactive because *ESF* cannot proceed to *ESFP* ($h_{3f} = h_{4f} = 0$) and it is only the free enzymes (i.e. that are not assembled into an *FDS*) that are active.

schematised in the usual concise way since there are certainly many more intermediate complexes involved in reality than indicated in the figures.

A two-partner model of *FDS* may be constructed as depicted in Fig. 2. There are two steps in the functioning of this *FDS* model: (*i*) the creation of the enzyme–enzyme bond as a consequence of the fact that enzyme $E$ has bound its substrate $S$ and (*ii*) the engagement of the bi-enzymatic *FDS* thus obtained in the catalysis of the overall reaction of the initial substrate, $S$, to the final product, $Q$. If substrate $S$ were to be entirely consumed in the reaction medium, then obviously the process of *FDS* formation would reverse and cause this structure to break down and release the free enzymes $E$ and $F$. When the concentration of $S$ is not zero, the relative concentrations of free and assembled enzymes depend on the values of the rate constants. Such a type of *FDS* as described in Fig. 2 is termed *catalytic*, in the sense that the formation of the enzyme assembly facilitates the progress of the overall reaction from $S$ to $Q$. Note that in this and the following figures, *ESF* is a formal description of the *functioning-dependent structure*, meaning that the bi-enzymatic complex *EF* is also bound to a substrate molecule: it does not mean that the substrate is a component of the bond of $E$ with $F$.

An *inhibitory FDS* (Fig. 3) may also be envisaged. In such an *FDS*, enzymes again assemble as in Fig. 2, but only the free enzyme $F$ can catalyse the reaction of $P$ to $Q$ and the complex *ESF* has no catalytic effect.

## 3. Steady-state kinetics

### 3.1. Statement of the problem

In the following, the concentration of any substance, $X$, will be symbolised $[X]$. To compare the steady-state kinetic behaviour of the catalytic and inhibitory *FDSs* (Figs. 2 and 3, respectively) with that of the similar, 'non-assembled' enzymes that do not form an *FDS* (Fig. 1), we use the simplifying assumptions that ($a_1$) the reaction medium is homogeneous, ($a_2$) the channelling of $P$ from $E$ to $F$ within the *FDS* is perfect (i.e., there is no liberation of $P$ into the reaction medium), ($a_3$) all the reactions of formation or decay of complexes other than those indicated in the figures, for instance

$$ES + FP \rightleftharpoons ESFP \tag{2}$$

are negligible, ($a_4$) the free enzymes $E$ and $F$ are of the Michaelis–Menten type (i.e. apart from their possible changes in their structure due to their assembling into an *FDS*, there are no allosteric transitions due to the fixation/release of regulatory ligands), ($a_5$) all the stoichiometric coefficients involved in the reactions are taken to be equal to 1 and ($a_6$) under steady-state conditions, $[S]$ is maintained at a constant value, $[S]_0$, and $[Q]$ at a zero value.

In the conditions modelled here, the forward rate constants ($k_{jf}$ and $h_{jf}$) are expressed in $\mathrm{mol}^{-1}\,\mathrm{s}^{-1}\,\mathrm{m}^3$ while the reverse rate constants ($k_{jr}$ and $h_{jr}$) are expressed in $\mathrm{s}^{-1}$. Moreover, for easier analysis, we treat the problem using dimensionless variables and parameters, i.e. dimensionless rate constants $\alpha_j$ and $\beta_j$ (corresponding to $k_j$ and $h_j$, respectively), the dimensionless time, $\tau$, and dimensionless concentrations (written using lower-case letters). The definitions of these dimensionless quantities are given in Appendix A.

The equilibrium constant, $K$, of the overall reaction of $S$ to $Q$ is independent of the way in which this reaction is catalysed (that is, via non-assembled enzymes or via a catalytic or inhibitory *FDS*). This imposes constraints on the rate constants, the consequence of which is that two of the rate constants (e.g., $\alpha_{4f}$ and $\beta_{4f}$) cannot be chosen arbitrarily in the modelling process, but have to be calculated as functions of the equilibrium constant and the other rate constants. The expressions of the equilibrium constant, $K$, and of $\alpha_{4f}$ and $\beta_{4f}$ are given in Appendix B. Moreover, when not at equilibrium, the overall reaction will tend to transform $S$ into $Q$ when $[S]/[Q] > 1/K$, while it will tend to transform $Q$ into $S$ when $[S]/[Q] < 1/K$. With $[Q] = 0$ according to assumption ($a_6$), the reaction will always proceed from $S$ to $Q$.

### 3.2. Steady-state kinetic behaviour of the various two-partner systems

The derivation of the expression of the steady-state rate of functioning, $u$, of the overall reaction of $S$ to $Q$ as a function of the concentration of substrate, $s_0$, in the case of non-assembled enzymes is given in Appendix C. Note that, in this and the following appendices, we have written sets of *independent* equations, eliminating some time derivatives (e.g., $\mathrm{d}e/\mathrm{d}\tau$ and $\mathrm{d}f/\mathrm{d}\tau$ in Appendix C, $\mathrm{d}e_{fs}/\mathrm{d}\tau$ in Appendix D, and $\mathrm{d}e_s/\mathrm{d}\tau$ and $\mathrm{d}f/\mathrm{d}\tau$ in Appendix E) as a consequence of the mass-conservation relations. Fig. 4 gives an example of the results that have been computed with a particular choice of the parameters (equilibrium and rate constants). With the many different values of the parameters we have tested, we have always obtained the same type of banal behaviour, in which $u$ increases monotonically as a function of $s_0$ until reaching a saturation plateau.

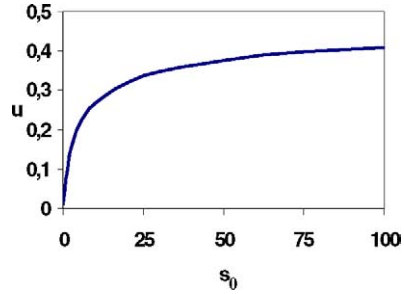


Fig. 4. Steady-state reaction rate, $u$, computed as a function of the concentration of substrate, $s_0$, for a two-enzyme system in the case of free enzymes (i.e. enzymes not assembled into an *FDS*). The parameter values are: $K = 10$, $x_E = x_F = 0.5$, $q = 0$, $\alpha_{1f}$ to $\alpha_{3f} = 1$, $\alpha_{1r}$ to $\alpha_{4r} = 1$, $\alpha_{4f}$ calculated by Eq. (B.4). The computed value of the saturation plateau is 0.5.

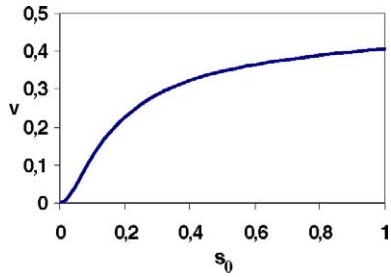


Fig. 5. Steady-state reaction rate, $v$, computed as a function of the concentration of substrate, $s_0$, for a two-enzyme, catalytic *FDS*. The parameter values are: $K = 10$, $x_E = x_F = 0.5$, $q = 0$, $\alpha_{1f}$ and $\alpha_{1r} = 1$, $\beta_{2f} = \beta_{2r} = 1$, $\beta_{3f} = 100$, $\beta_{3r} = 0.01$, $\beta_{4f}$ calculated by Eq. (B.5), $\beta_{4r} = 1$. The computed value of the saturation plateau is 0.5.

The expression of the steady-state rate of functioning of a catalytic *FDS*, $v$, is derived in Appendix D. When computing the dependence of $v$ on the concentration of initial substrate, $s_0$, there are choices of parameters with which we obtain the same banal type of behaviour (monotonically increasing curve up to a saturation plateau), as has been observed in the case of non-assembled enzymes. However, with

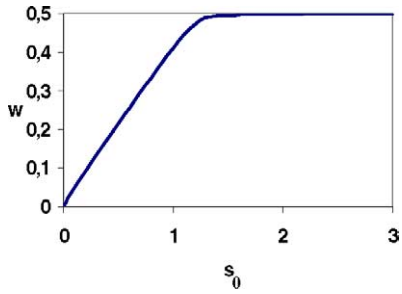


Fig. 6. Steady-state reaction rate, $w$, computed as a function of the concentration of substrate, $s_0$, for a two-enzyme, inhibitory *FDS*. The parameter values are: $K = 10$, $x_E = x_F = 0.5$, $q = 0$, $\alpha_{1f} = 1$, $\alpha_{2f} = 0.1$, $\alpha_{3f} = 10$, $\alpha_{1r} = 1$, $\alpha_{2r} = 10$, $\alpha_{3r} = 0.1$, $\alpha_{4r} = 1$, $\beta_{2f} = 1$, $\beta_{2r} = 1$, $\alpha_{4f}$ calculated by Eq. (B.4). The computed value of the saturation plateau is 0.5.

other choices of parameters (such as that indicated in Fig. 5), we find a more interesting behaviour in which the curve $\{s_0, v\}$ exhibits a sigmoidal shape, although none of the individual enzymes, $E$ and $F$, possesses any cooperativity per se (assumption $a_4$). In the present case of a two-partner enzyme-assembly, the sigmoidal character of the curve is not very pronounced, but, according to preliminary calculations with $n$-partner enzyme-assemblies, it seems that increasing the number, $n$, of partners in the enzyme-assemblies tends to increase the sigmoidal character of the $\{s_0, v\}$ curves (not shown). However that may be, our simulations suggest that the structuring of enzymes into a dynamic *FDS* while accomplishing their function may cause the emergence of a property characteristic of regulated systems (sigmoidal behaviour) that the free enzymes do not possess.

The equations governing the kinetic behaviour of an inhibitory *FDS* are given in Appendix E. When computing the dependence of the reaction rate of the inhibitory *FDS*, $w$, on the concentration of initial substrate, $s_0$, according to the equations given in the appendix, there are choices of the parameters (equilibrium and rate constants) with which again we obtain the same banal type of behaviour (monotonically increasing curve up to a saturation plateau) as shown in Fig. 4 with non-assembled enzymes. However, there are also choices of parameters where the presence of the inhibitory *FDS* tends to linearise the $\{s_0, w\}$ curve over a large range of $s_0$ values: for instance, Fig. 6 gives an example of a case in which the $\{s_0, w\}$ curve is linear almost up to the saturation plateau.

## 4. Discussion and conclusion

Apart from the obvious advantages of enzymes assembling into hyperstructures (see Introduction), it has been shown here that enzymes of the simple Michaelis–Menten type may display a richer (e.g., sigmoidal or linear) kinetic behaviour when they are engaged in *functioning-dependent structures* than when they remain non-assembled. Hence, under the highly structured conditions likely to exist in vivo, not only allosteric proteins, but also any sort of enzyme may exhibit regulatory properties provided it can form part of an *FDS*. It is also noteworthy that certain of the properties of the *FDSs* such as linear and sigmoid responses resemble the regulatory linear responses and step functions built into artificial electronic devices.

The possible occurrence of sigmoidal responses with *FDSs* is also reminiscent of apparent allosteric effects emerging in membrane-constrained co- or counter-transport proteins when the usual assumptions of very fast binding and release are relaxed [12].

The likely relevance of the concept of *functioning-dependent structure* to enzyme behaviour means, we suggest, that the classical *structure* → *function* relationship in biochemistry should be complemented by a reciprocal *function* → *structure* relationship. In other words, subcellular processes exist in which transient functioning structures are created and maintained by the very fact that they are accomplishing a function (see, e.g., [6,11]). This two-way relationship may prove to occur relatively frequently in living systems, while it is not generally encountered in non-living, physical, or chemical processes. Moreover, the assembly and decay of *functioning-dependent structures* in a living system will be responsible for an extra production of entropy, in addition to that arising from the normal reactions and transport processes in cells. This extra production of entropy by *FDSs* thus may be of particular relevance to living systems.

Here we have considered only very simple, two-partner *FDS* models, the steady-state kinetics of which has been studied by use of relatively straightforward calculation methods. In real living systems, however, much more complicated transient associations of proteins may occur, involving multi-partner associations and possibly forming dynamic networks of *FDSs*. We speculate that the regulatory properties of such complex *FDSs* will prove to be even more numerous and

clear-cut than in the simple cases examined here. At present, the difficulty of modelling such complex systems is considerable but may become feasible with the use of appropriate mathematical and computer techniques. Despite this difficulty, the simple approach adopted in this paper shows that the *FDS* concept has interesting implications and that more complex and realistic *FDS* models should be envisaged.

## Appendix A. Definition of dimensionless quantities

Dimensionless quantities have been defined by normalising all concentrations to the sum of the total concentrations of $E$ and $F$, $[E]_t+[F]_t$, and all time values to $1/k_{1r}$. As a consequence, the molar fractions of enzymes $E$ and $F$ are:

$$x_E = [E]_t/\big([E]_t+[F]_t\big), \quad x_F = [F]_t/\big([E]_t+[F]_t\big) \quad \text{with } x_E + x_F = 1 \tag{A.1}$$

the dimensionless concentrations of all the substances involved are:

$$s = [S]/\big([E]_t+[F]_t\big), \quad p = [P]/\big([E]_t+[F]_t\big)$$
$$q = [Q]/\big([E]_t+[F]_t\big) \tag{A.2}$$

$$e = [E]/\big([E]_t+[F]_t\big), \quad f = [F]/\big([E]_t+[F]_t\big) \tag{A.3}$$

$$e_s = [ES]/\big([E]_t+[F]_t\big)$$
$$f_p = [FP]/\big([E]_t+[F]_t\big) \tag{A.4}$$

$$ef_s = [ESF]/\big([E]_t+[F]_t\big)$$
$$ef_{sp} = [ESFP]/\big([E]_t+[F]_t\big) \tag{A.5}$$

the dimensionless time, $\tau$, is:

$$\tau = tk_{1r} \tag{A.6}$$

the dimensionless reverse rate constants are:

$$\alpha_{jr} = k_{jr}/k_{1r}, \quad \beta_{jr} = h_{jr}/k_{1r} \tag{A.7}$$

with, obviously:

$$\alpha_{1r} = k_{1r}/k_{1r} \equiv 1 \tag{A.8}$$

and the dimensionless forward rate constants are:

$$\alpha_{jf} = (k_{jf}/k_{1r})\big([E]_t+[F]_t\big)$$
$$\beta_{jf} = (h_{jf}/k_{1r})\big([E]_t+[F]_t\big) \tag{A.9}$$

## Appendix B. Equilibrium constant and non-independent rate constants

The (dimensionless) equilibrium constant of the overall reaction (Eq. (1)):

$$K = [Q]_{eq}/[S]_{eq} = q_{eq}/s_{eq} \tag{B.1}$$

in which $[S]_{eq}$ and $[Q]_{eq}$ are the equilibrium concentrations of $S$ and $Q$ (and $s_{eq}$ and $q_{eq}$ the corresponding dimensionless quantities), may be calculated both in the case of enzymes not assembled in a *FDS* and in the case when a catalytic *FDS* occurs. This is written:

$$q_{eq}/s_{eq} = (\alpha_{1f}\alpha_{2r}\alpha_{3f}\alpha_{4r})/(\alpha_{1r}\alpha_{2f}\alpha_{3r}\alpha_{4f}) = K \tag{B.2}$$

and

$$q_{eq}/s_{eq} = (\beta_{3f}\beta_{4r})/(\beta_{3r}\beta_{4f}) = K \tag{B.3}$$

respectively. As a consequence, not all the rate constants are independent from one another, but two of them are functions of the other rate constants and the equilibrium constant, e.g.:

$$\alpha_{4f} = (\alpha_{1f}\alpha_{2r}\alpha_{3f}\alpha_{4r})/(K\alpha_{1r}\alpha_{2f}\alpha_{3r}) \tag{B.4}$$

and

$$\beta_{4f} = (\beta_{3f}\beta_{4r})/(K\,\beta_{3r}) \tag{B.5}$$

## Appendix C. Steady-state reaction rate, $u$, in the case of enzymes not assembled in a *FDS*

With non-assembled enzymes (Fig. 1), the mass-conservation equations are:

$$x_E = e + e_s, \quad x_F = f + f_p \tag{C.1}$$

a set of independent equations governing the system under steady-state conditions is:

$$dp/d\tau = -\alpha_{2f}pe + \alpha_{2r}e_s - \alpha_{3f}pf + \alpha_{3r}f_p = 0 \tag{C.2}$$
$$de_s/d\tau = \alpha_{1f}se - \alpha_{1r}e_s + \alpha_{2f}pe - \alpha_{2r}e_s = 0 \tag{C.3}$$
$$df_p/d\tau = \alpha_{3f}pf - \alpha_{3r}f_p + \alpha_{4f}qf - \alpha_{4r}f_p = 0 \tag{C.4}$$

with, according to assumption $a_6$:

$$s = s_0, \quad q = 0 \tag{C.5}$$

and the initial conditions are:

$$p(0) = 0, \quad e_s(0) = 0, \quad f_p(0) = 0 \tag{C.6}$$

The expressions of the variables ($e$, $f$, $e_s$, $f_p$, and $p$) are easily found to be:

$$e_s = x_E(\alpha_{1f}s_0 + \alpha_{2f}p)/(\alpha_{1f}s_0 + \alpha_{1r} + \alpha_{2f}p + \alpha_{2r}) \quad (C.7)$$

$$f_p = x_F(\alpha_{3f}p)/(\alpha_{3f}p + \alpha_{3r} + \alpha_{4r}) \quad (C.8)$$

$$e = x_E - e_s \quad (C.9)$$

$$f = x_F - f_p \quad (C.10)$$

$$p = \left(-Ds_0 - G - \left((Ds_0 + G)^2 - 4AHs_0\right)^{1/2}\right)/2A \quad (C.11)$$

with $A$, $D$, $G$ and $H$ being expressed as:

$$A = -\alpha_{2f}\alpha_{3f}(\alpha_{1r}x_E + \alpha_{4r}x_F) \quad (C.12)$$

$$D = \alpha_{1f}\alpha_{3f}(\alpha_{2r}x_E - \alpha_{4r}x_F) \quad (C.13)$$

$$G = -\alpha_{1r}\alpha_{2f}(\alpha_{3r} + \alpha_{4r})x_E - \alpha_{3f}\alpha_{4r}(\alpha_{1r} + \alpha_{2r})x_F \quad (C.14)$$

$$H = \alpha_{1f}\alpha_{2r}(\alpha_{3r} + \alpha_{4r})x_E \quad (C.15)$$

and the reaction rate, $u$ (which corresponds to both the consumption of $S$ and the production of $Q$), is written:

$$u = \alpha_{1f}s_0e - \alpha_{1r}e_s = \alpha_{4r}f_p \quad (C.16)$$

## Appendix D. Steady-state reaction rate, $v$, in the case of a catalytic *FDS*

The mass-conservation equations of the catalytic *FDS* (Fig. 2) are:

$$x_E = e + e_s + ef_s + ef_{sp}, \quad x_f = f + ef_s + ef_{sp} \quad (D.1)$$

Then three independent steady-state equations are derived in a manner similar to that in the case with non-assembled enzymes, e.g.:

$$de/d\tau = -\alpha_{1f}se + \alpha_{1r}e_s = 0 \quad (D.2)$$

$$de_s/d\tau = \alpha_{1f}se - \alpha_{1r}e_s + \beta_{2r}ef_s - \beta_{2f}fe_s = 0 \quad (D.3)$$

$$def_{sp}/d\tau = \beta_{3f}sef_s - \beta_{3r}ef_{sp} + \beta_{4f}qef_s - \beta_{4r}ef_{sp} = 0 \quad (D.4)$$

with the conditions:

$$s = s_0, \quad q = 0, \quad p(0) = 0, \quad e_s(0) = 0, \quad ef_s(0) = 0, \quad ef_{sp}(0) = 0 \quad (D.5)$$

Defining $P1$, $P2$, $P3$, $A$, $B$ and $C$ as:

$$P1 = (\alpha_{1f}\beta_{2f}s_0)/(\alpha_{1r}\beta_{2r})$$
$$P2 = 1 + \left((\alpha_{1f}s_0)/\alpha_{1r}\right) \quad (D.6)$$
$$P3 = 1 + \left((\beta_{3f}s_0)/(\beta_{3r} + \beta_{4r})\right)$$

$$A = P1P2P3, \qquad B = P2 - \left(P1P3(x_E - x_F)\right)$$
$$C = -x_E \quad (D.7)$$

the variables of the problem are expressed as

$$e = \left(-B + (B^2 - 4AC)^{1/2}\right)/2A \quad (D.8)$$

$$f = \left(1 + \left((\alpha_{1f}s_0)/\alpha_{1r}\right)\right)e - x_E + x_F \quad (D.9)$$

$$e_s = (\alpha_{1f}s_0e)/\alpha_{1r} \quad (D.10)$$

$$ef_s = \left((\alpha_{1f}\beta_{2f}s_0)/(\alpha_{1r}\beta_{2r})\right) \times \left(\left(1 + (\alpha_{1f}s_0)/\alpha_{1r}\right)e^2 - (x_E - x_F)e\right) \quad (D.11)$$

$$ef_{sp} = \left((\beta_{3f}s_0)/(\beta_{3r} + \beta_{4r})\right)ef_s \quad (D.12)$$

and the reaction rate, $v$ (again corresponding to both the consumption of $S$ and the production of $Q$), is written:

$$v = \alpha_{1f}es - \alpha_{1r}e_s + \beta_{3f}ef_s - \beta_{3r}ef_{sp} = \beta_{4r}ef_{sp} \quad (D.13)$$

## Appendix E. Steady-state reaction rate, $w$, in the case of an inhibitory *FDS*

In the case of an inhibitory *FDS* (Fig. 3), the mass-conservation equations are:

$$x_E = e + e_s + ef_s, \quad x_f = f + f_p + ef_s \quad (E.1)$$

and a set of independent steady-state equations is written:

$$de/d\tau = -\alpha_{1f}se + (\alpha_{1r} + \alpha_{2r})e_s - \alpha_{2f}pe = 0 \quad (E.2)$$

$$def_s/d\tau = \beta_{2f}fe_s - \beta_{2r}ef_s = 0 \quad (E.3)$$

$$df_p/d\tau = \alpha_{3f}pf - (\alpha_{3r} + \alpha_{4r})f_p + \alpha_{4f}qf = 0 \quad (E.4)$$

$$dp/d\tau = -\alpha_{2f}pe + \alpha_{2r}e_s - \alpha_{3f}pf + \alpha_{3r}f_p = 0 \quad (E.5)$$

with

$$s = s_0, \quad q = 0, \quad p(0) = 0, \quad e_s(0) = 0, \quad ef_s(0) = 0, \quad f_p(0) = 0 \quad (E.6)$$

Four of the variables ($f$, $e_s$, $f_p$ and $ef_s$) can be expressed as functions of the other two ($p$ and $e$), i.e.:

$$e_s = \big((\alpha_{1f}s_0 + \alpha_{2f}p)/(\alpha_{1r} + \alpha_{2r})\big)e \tag{E.7}$$

$$f = \big((\alpha_{3r} + \alpha_{4r})(\alpha_{1f}\alpha_{2r}s_0 - \alpha_{1r}\alpha_{2f}p)/\big(\alpha_{3f}\alpha_{4r}(\alpha_{1r} + \alpha_{2r})\big)\big)e \tag{E.8}$$

$$f_p = \big((\alpha_{1f}\alpha_{2r}s_0 - \alpha_{1r}\alpha_{2f}p)/\big(\alpha_{4r}(\alpha_{1r} + \alpha_{2r})\big)\big)e \tag{E.9}$$

$$ef_s = \big((\beta_{2f}(\alpha_{3r} + \alpha_{4r})(\alpha_{1f}s_0 + \alpha_{2f}p) \times (\alpha_{1f}\alpha_{2r}s_0 - \alpha_{1r}\alpha_{2f}p)\big)/\big(\alpha_{3f}\alpha_{4r}\beta_{2r}(\alpha_{1r} + \alpha_{2r})^2 p\big)\big)e^2 \tag{E.10}$$

Defining $A$, $B_1$, $C_1$, $B_2$ and $C_2$ as:

$$ef_s = Ae^2, \quad e + e_s = B_1 e, \quad -x_E = C_1$$
$$f + f_p = B_2 e, \quad -x_F = C_2 \tag{E.11}$$

Eqs. (E.1) may be rewritten:

$$Ae^2 + B_1 e + C_1 = 0 \tag{E.12}$$

and

$$Ae^2 + B_2 e + C_2 = 0 \tag{E.13}$$

Since concentrations and rate constants are by nature positive quantities, $C_1$ and $C_2$ are negative and $B_1$ is positive. Since the overall reaction proceeds in the direction $S \to P \to Q$ (as a consequence of $q$ being maintained equal to zero), the factor $(\alpha_{1f}\alpha_{2r}s_0 - \alpha_{1r}\alpha_{2f}p)$ in the expressions of $A$ and $B_2$ is positive and $A$ and $B_2$ thus are also positive. Therefore, each of Eqs. (E.12) and (E.13) has only a single solution, $e_1$ and $e_2$, respectively, that is biologically relevant, i.e.

$$e_1 = \big(-B_1 + \big(B_1^2 - 4AC_1\big)^{1/2}\big)/2A \tag{E.14}$$

$$e_2 = \big(-B_2 + \big(B_2^2 - 4AC_2\big)^{1/2}\big)/2A \tag{E.15}$$

where $e_1$ and $e_2$ are functions of only the variable $p$. There is then an easy numerical solution to the problem that is obtained by systematically varying $p$ until the correct $p$ value is obtained for which:

$$e_1 = e_2 = e \tag{E.16}$$

at the desired precision. The other variables ($f$, $e_s$, $f_p$ and $ef_s$) then are calculated from these values of $p$ and $e$ by using Eqs. (E.7) to (E.10) and the reaction rate, $w$ (again corresponding to both the consumption of $S$ and the production of $Q$), is written:

$$w = \alpha_{1f}s_0 e - \alpha_{1r}e_s = \alpha_{4r}f_p \tag{E.17}$$

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