

# Retroactivity Analysis of a Chemical Reaction Network Module for the Subtraction of Molecular Fluxes

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**Abstract**—The availability of a well-characterised subtraction module is a key step towards the realisation of modular embedded feedback controllers in synthetic biological systems. A well-known problem when dealing with complex biosystems is represented by the retroactivity effect, which can significantly modify the dynamics of interconnected subsystem, with respect to the behaviour they exhibit when disconnected from each other. In this paper, we illustrate a minimal CRN that implements a subtraction operation between two input molecular fluxes. In order to assess its effectiveness as a module of a more complex system, we analyse its retroactivity upon interconnection. More specifically, we connect the subtraction module with an upstream module, which determines the dynamics of the inputs species, and with a downstream transcriptional module, which acts as a load. By comparing the dynamics of the loaded and unloaded subtractor, we show that the retroactivity can be attenuated when the dynamics of the subtractor and of the load system evolve over different timescales. This result, obtained through a singular perturbation analysis, is confirmed by means of numerical simulations.

## I. INTRODUCTION

Achieving biological behaviours with novel and predictable functionalities is a central topic in Synthetic Biology, which is strictly linked to the theoretical and technological challenges posed by the design of molecular feedback controllers. The control of biomolecular systems is largely based on the capability of engineering different molecular components, e.g. plasmids, DNA or RNA strands, to create biological devices that can accomplish specific tasks (see, e.g., [1],[2]). Furthermore, it is also important to devise engineering methods to assemble such components in a modular, scalable manner, such that the resulting systems exhibit a predictable behaviour.

In previous works (see [3],[4]), we have proposed a simple design of an embedded synthetic feedback controller, based on the Chemical Reaction Networks (CRN) formalism. A feature of our approach consists in the definition of molecular fluxes as input/output signals that connect the different subsystems. This choice is particularly suited to devise a modular approach to the design of complex systems; moreover, the use of molecular fluxes naturally arises in certain contexts, e.g. in metabolic engineering, which aims at the regulation of the fluxes of one or more species for industrial applications (see [5],[6]). The present paper deals with the analysis of a CRN that outputs the difference

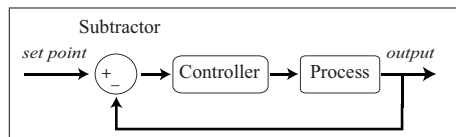


Fig. 1. Block diagram of the classical feedback control scheme: the subtractor module allows the output of the controlled process to be compared with the desired value (set point) at each time instant; the difference between these two quantities is the error signal, which constitutes the input to the controller.

between two input fluxes; this is a basic component of the classical feedback control scheme, illustrated in Fig. 1, which is required to compare the desired set-point with the actual output of the process to be controlled. In order to evaluate the exploitability of the proposed *molecular subtractor* as a component of a more complex system, here we will focus on the analysis of its retroactivity properties [7], [8], [9]. Along the lines of [10], [11], we consider the interconnection of the subtractor with a generic upstream module, which produces and consumes the two input species, and with a downstream transcriptional module. The final goal is to show how the retroactivity of the subtractor can be arbitrarily attenuated provided that the connected modules evolve over different timescales.

The paper is organized as follows: in Section II we discuss the general properties required from a subtraction block and translate these into a minimal CRN-based subtraction module. Section III briefly recalls the concept of retroactivity and a general modeling scheme for the connection of biomolecular systems. In Section IV the behaviour of the subtractor under loaded and unloaded conditions is investigated, exploiting a nonlinear analysis method, namely singular perturbations [12], and numerical simulations. Finally, Section V, provides some concluding remarks.

## II. SUBTRACTION MODULE BASED ON CHEMICAL REACTION NETWORK

In this section we illustrate the general properties that a CRN must satisfy in order to compute the difference between two molecular fluxes and provide a mathematical description of a minimal CRN that implements this function.

### A. Minimal properties for a CRN-based subtractor function

The realization of the subtractor given below is minimal in the sense that it involves the minimum number of molecular species required for implementing a CRN that satisfies properties P1)-P2). A generic CRN comprises two molecular species,  $A$  and  $B$ , whose fluxes  $\Phi_A(t)$  and  $\Phi_B(t)$

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are the input signals to be subtracted, and a third species  $C$ , whose flux  $\Phi_C(t)$  is the output signal. Assume that  $\Phi_A(t) > \Phi_B(t)$ : the relationship  $\Phi_C = \Phi_A - \Phi_B$  requires that

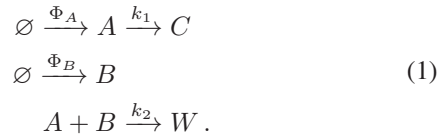
P1) For each molecule of  $B$  that enters the CRN, exactly one molecule of  $A$  is converted into a species different from  $C$  (it may also be the *null* species, i.e. the molecule is degraded);

P2) Eventually, the molecules of  $A$  that are not converted into other species are turned into molecules of  $C$ .

Notice that, without the minimality constraints, there would be an infinite number of CRNs that satisfy properties P1) and P2): indeed, in that case the conversion of  $A$  into other molecules (either  $C$  or non- $C$ ), might occur through any sequence of reactions, involving any number of intermediate species.

### B. CRN-based subtraction module

To realize the subtraction operation between the fluxes  $\Phi_A$  and  $\Phi_B$ , we propose to employ the CRN



The model of CRN (1) is given by

$$\dot{a} = \Phi_A - k_1 a - k_2 a b \quad (2a)$$

$$\dot{b} = \Phi_B - k_2 a b \quad (2b)$$

$$\dot{w} = k_2 a b \quad (2c)$$

$$\Phi_C = \dot{c} = k_1 a, \quad (2d)$$

where italic lowercase letters,  $a$ ,  $b$ ,  $c$  and  $w$  are used to denote the concentration of species  $A$ ,  $B$ ,  $C$  and  $W$ , respectively. A subtraction function is possible thanks to a conversion into species  $C$  of that molecules of  $A$  which don't bind to  $B$ , therefore species  $C$  represent the difference between  $A$  and  $B$  while any species  $A$  bounded to  $B$  undergo a conversion into species  $W$  which cannot longer bind to  $B$ .

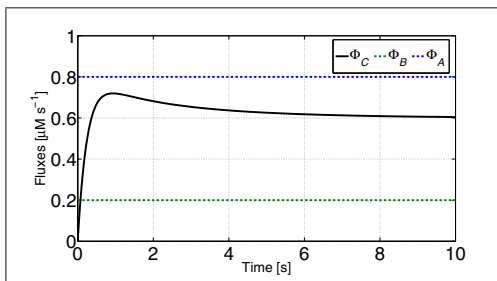


Fig. 2. Response of the isolated subtractor (CRN (1)) with the input fluxes given by  $\Phi_A = 0.8 \mu M s^{-1}$  and  $\Phi_B = 0.2 \mu M s^{-1}$ , and the kinetic parameters set to  $k_1 = 4 s^{-1}$  and  $k_2 = 3 (\mu M s)^{-1}$  to have a settling time by 10 s. The output flux (solid line)  $\Phi_C$  converges to the difference between the two input fluxes (dashed lines).

Note that CRN (1) satisfies properties P1)-P2) and, as shown in the simulation reported in Figure 2, the output flux  $\Phi_C$  converges to the difference between the inputs fluxes  $\Phi_A$  and  $\Phi_B$ .

### III. RETROACTIVITY TO THE INPUT AND TO THE OUTPUT

In this section we introduce the concept of *retroactivity*, which is a way to quantify how the interconnection of two systems affects the dynamics of each of them with respect to their behaviour when isolated.

#### A. Mathematical model for the connection of bio-molecular systems

Let us consider a generic system  $S$ , shown in Figure 3, with  $u \in D_u \subset \mathbb{R}_+^q$ ,  $x \in D_x \subset \mathbb{R}_+^n$  and  $v \in D_v \subset \mathbb{R}_+^p$  denoting concentrations of chemical species, such as proteins, enzymes, DNA sites, etc. Let  $r(x, u)$  and  $s(x, v)$  be reaction rate vectors modeling *retroactivity to the input* and *retroactivity to the output*, respectively, whereas  $g(u, t)$  and  $f(x, u)$  are reaction rate vectors representing the input and output fluxes of system  $S$ .

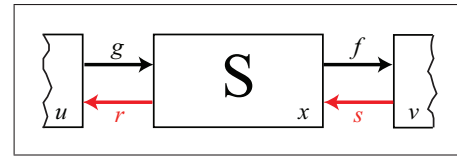


Fig. 3. A system  $S$  with its input and output signals. The red signals denote signals originating from retroactivity upon interconnection of  $S$  with other modules.

Let  $\mathcal{A} \in \mathbb{R}^{r \times q}$ ,  $\mathcal{B} \in \mathbb{R}^{r \times n}$ ,  $\mathcal{C} \in \mathbb{R}^{s \times n}$  and  $\mathcal{D} \in \mathbb{R}^{s \times p}$  be constant matrices. Let  $l(v) \in \mathbb{R}^p$ ,  $h(v, t) \in \mathbb{R}^p$  be vector fields and  $G_\alpha$ ,  $G_\beta$  be positive constants modeling the timescale of the interconnection mechanism of  $S$ . The formal mathematical description of the interconnected system depicted in Figure 3 then reads

$$\dot{u} = g(u, t) + G_\alpha \mathcal{A} r(x, u) \quad (3a)$$

$$\dot{x} = G_\alpha \mathcal{B} r(x, u) + G_\alpha f(x, u) + G_\beta \mathcal{C} s(x, v) \quad (3b)$$

$$\dot{v} = G_\beta \mathcal{D} s(x, v) + G_\beta l(v) + h(v, t). \quad (3c)$$

Note that a mathematical description of the unloaded system can be readily obtained by considering  $s(x, v) = 0$ .

### IV. ANALYSIS AND ATTENUATION OF THE RETROACTIVITY

To study the retroactivity properties of our CRN subtractor module, we will now compare the dynamics of the loaded and of the unloaded system, using a singular perturbation analysis. This approach allows us to deal with subsystems evolving over different timescales; in particular, by separating the fast and slow dynamics, it is possible to reduce the complexity of the analysis, by approximating the global dynamics with that of the slow subsystem. To show how to describe dynamics by singular perturbation analysis, we illustrate in details the loaded system which is more involved and then present briefly the more simple unloaded case.

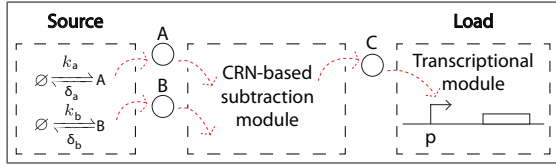


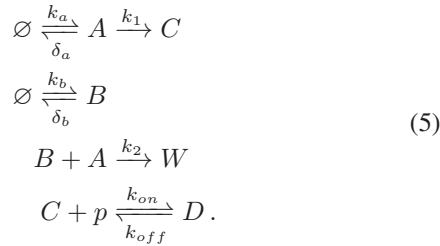
Fig. 4. The analysis of the retroactivity is realised by a modular interconnection of the CRN-based subtraction module. The CRN-based subtraction module takes two fluxes  $A$  and  $B$  as inputs arising from a generic upstream module mathematically described with reaction rates of formation and degradation of each input, and produces  $C$  as output flux. The transcription factor  $C$  binds to operator sites on the promoter  $p$  in order to produce a complex protein promoter  $D$ .

### A. Singular perturbation analysis of the loaded CRN-based-subtractor

Adopting a similar approach to that in [11], we analyse the retroactivity for the minimal CRN-based subtractor connected to the following downstream transcriptional module,



which takes as input the flux of  $C$  produced by the subtractor. Moreover, to study the retroactivity for both inputs, we consider two source modules, characterised by two generic rate of formation ( $k_a(t)$  and  $k_b(t)$ ) and two degradation coefficients ( $\delta_a$  and  $\delta_b$ ) for the two inputs. The interconnected CRN system is reported in Figure 4 and corresponds to the following reaction network



Taking into account the conservation law  $p_{TOT} = p + d$ , the dynamics of CRN (5) are described by

$$\dot{a} = k_a(t) - \delta_a a - k_1 a - k_2 a b \quad (6a)$$

$$\dot{b} = k_b(t) - \delta_b b - k_2 a b \quad (6b)$$

$$\dot{w} = k_2 a b \quad (6c)$$

$$\dot{c} = k_1 a + k_{off} d - k_{on}(p_{TOT} - d) c \quad (6d)$$

$$\dot{d} = -k_{off} d + k_{on}(p_{TOT} - d) c. \quad (6e)$$

To write system (6) in terms of non-dimensional variables, we define  $\bar{k}_A := \frac{max_t k_a(t)}{\delta_a}$ ,  $\bar{k}_B := \frac{max_t k_b(t)}{\delta_b}$ ,  $u_A := \frac{a}{\bar{k}_A}$ ,  $u_B := \frac{b}{\bar{k}_A}$ ,  $x_1 := \frac{w}{\bar{k}_A}$ ,  $x_2 := \frac{c}{\bar{k}_A}$ ,  $\nu := \frac{d}{\bar{k}_A}$ ,  $\tilde{k}_A(t) := \frac{k_a(t)}{\delta_a \bar{k}_A}$ ,  $\tilde{k}_B(t) := \frac{k_b(t)}{\delta_b \bar{k}_A}$  and  $\tau = \delta t$  so that for a variable  $x$  we denote  $\dot{x} := \frac{dx}{d\tau}$ . Moreover, by assuming the parameter  $k_{off}$  much larger than  $k_1$  and  $k_2$  which is in turn much larger than  $\delta_a$  and  $\delta_b$ , the timescale differences can be made explicit by defining the large parameters  $G_1 := \frac{k_1}{\delta_a}$ ,  $G_2 := \frac{k_2 \bar{k}_A}{\delta_a}$  and  $G_3 := \frac{k_{off}}{\delta_a}$  in which  $G_3 \gg G_2 \gg G_1 \gg 1$ . Letting the

dissociation constant  $k_D := \frac{k_{off}}{k_{on}}$ , system (6) can then be described as

$$\dot{u}_A = \tilde{k}_A(t) - u_A - G_1 u_A - G_2 u_A u_B \quad (7a)$$

$$\dot{u}_B = \tilde{k}_B(t) - \frac{\delta_b}{\delta_a} u_B - G_2 u_A u_B \quad (7b)$$

$$\dot{x}_1 = G_2 u_A u_B \quad (7c)$$

$$\dot{x}_2 = G_1 u_A + G_3 \left( \nu - \frac{x_2}{k_D} (p_{TOT} - \bar{k}_A \nu) \right) \quad (7d)$$

$$\dot{\nu} = -G_3 \left( \nu - \frac{x_2}{k_D} (p_{TOT} - \bar{k}_A \nu) \right). \quad (7e)$$

By performing a linear coordinate transformation to take system (7) into the new variables,  $z_1 = u_A + x_1 + x_2 + \nu$ ,  $z_2 = u_B + x_1$ ,  $y_1 = x_1$  and  $y_2 = x_2 + \nu$ , we easily obtain that  $u_A = z_1 - y_1 - y_2$  and  $u_B = z_2 - y_1$ . Now, defining  $\epsilon_1 = \frac{1}{G_2}$ ,  $\epsilon_2 = \frac{1}{G_1}$  and  $\epsilon_3 = \frac{1}{G_3}$  we can write the loaded system in the following singular perturbation form,

$$\dot{z}_1 = \tilde{k}_A(t) - z_1 + y_1 + y_2 \quad (8a)$$

$$\dot{z}_2 = \tilde{k}_B(t) - \frac{\delta_b}{\delta_a} (z_2 - y_1) \quad (8b)$$

$$\epsilon_1 \dot{y}_1 = (z_1 - y_1 - y_2) (z_2 - y_1) \quad (8c)$$

$$\epsilon_2 \dot{y}_2 = z_1 - y_1 - y_2 \quad (8d)$$

$$\epsilon_3 \dot{\nu} = -\left( \nu - \frac{y_2 - \nu}{k_D} (p_{TOT} - \bar{k}_A \nu) \right). \quad (8e)$$

Approximating the left-hand side of Eqs. (8c)-(8a) to zero, yields a system of algebraic equations, from which we can compute  $y_1$ ,  $y_2$  and  $\nu$  as functions of  $z = (z_1 \ z_2)^T$  from equations (8c)-(8e), which represent the fast subsystem. Substituting  $y_1 = \gamma_{y_1}(z)$  and  $y_2 = \gamma_{y_2}(z)$  into (8a)-(8b), it is possible to approximate system (8) with (8a)-(8b), which represent the slow subsystem, thus obtaining a reduced order system. For the sake of brevity, we here omit a rigorous mathematical analysis of the conditions under which such approximation holds, which will be given in future work.

### B. Singular perturbation analysis of the unloaded CRN-based-subtractor

To study the dynamics of the unloaded system, we analyse system (3) by considering  $s(x, \nu) = 0$ . Looking at CRN (5) without considering the downstream transcriptional module (4) the dynamics are described as follows,

$$\dot{a} = k_a(t) - \delta_a a - k_1 a - k_2 a b \quad (9a)$$

$$\dot{b} = k_b(t) - \delta_b b - k_2 a b \quad (9b)$$

$$\dot{w} = k_2 a b \quad (9c)$$

$$\dot{c} = k_1 a. \quad (9d)$$

By using the same non-dimensional variables we can perform a linear coordinate transformation to take system (9) into the new variables  $z_1 = u_A + x_1 + x_2$ ,  $z_2 = u_B + x_1$ ,  $y_1 = x_1$  and  $y_2 = x_2$ , and obtain that  $u_A = z_1 - y_1 - y_2$  and  $u_B = z_2 - y_1$ . Defining  $\epsilon_1 = \frac{1}{G_2}$ ,  $\epsilon_2 = \frac{1}{G_1}$  and  $\epsilon_3 = \frac{1}{G_3}$  we can write the

unloaded system in the singular perturbation form

$$\dot{z}_1 = \tilde{k}_A(t) - z_1 + y_1 + y_2 \quad (10a)$$

$$\dot{z}_2 = \tilde{k}_B(t) - \frac{\delta_b}{\delta_a}(z_2 - y_1) \quad (10b)$$

$$\epsilon_1 \dot{y}_1 = (z_1 - y_1 - y_2)(z_2 - y_1) \quad (10c)$$

$$\epsilon_2 \dot{y}_2 = z_1 - y_1 - y_2. \quad (10d)$$

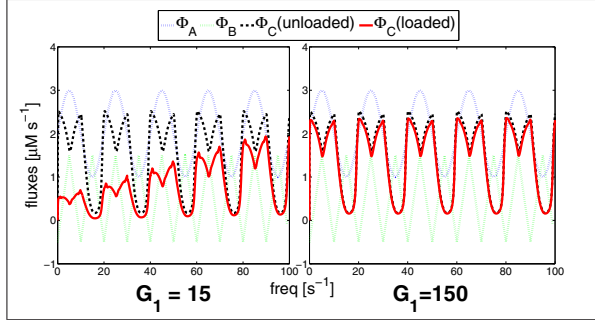


Fig. 5. Comparison of the loaded (red solid line) and unloaded (black dashed line) system response; the inputs are a sinusoidal signal  $\Phi_A = \sin(0.1\pi f) + 2$  (blue dotted line), and a sawtooth signal,  $\Phi_B = \text{sawtooth}(0.2\pi f) + 0.5$  (green dotted line). The timescale parameters are set to  $G_1 = 15$ ,  $G_2 = 50$  and  $G_3 = 200$  (in the left panel), and  $G_1 = 150$ ,  $G_2 = 500$  and  $G_3 = 10000$  (in the right panel): in the latter case  $G_3 \gg G_2 \gg G_1 \gg 1$  and the effect of retroactivity to the output is negligible, thus yielding similar behaviour of the loaded ( $p_{TOT} = 100\mu M$ ) and unloaded ( $p_{TOT} = 0$ ) systems.

### C. Comparison between the loaded and unloaded CRN-based-subtractor

Now, by comparing the singular perturbation forms (8) and (10), it is easy to identify that the reduced-order loaded system (8a)-(8b) is equal to the reduced-order unloaded system (10a)-(10b). Thus, as shown in Figure(5), the responses of the loaded and unloaded systems show negligible differences, thus the retroactivity to the output is attenuated. Finally, we can take both reduced-order systems back to their corresponding previous coordinates, using the expressions

$$\begin{aligned} \dot{z}_1 &= \dot{u}_A + \dot{\gamma}_{y_1}(z) + \dot{\gamma}_{y_2}(z) \\ \dot{z}_2 &= \dot{u}_B + \dot{\gamma}_{y_1}(z). \end{aligned} \quad (11)$$

Substituting  $z_1$  and  $z_2$  in the reduced-order loaded and unloaded systems yields, in both cases,

$$\begin{aligned} \dot{z}_1 &= \tilde{k}_A(t) - z_1 + \gamma_{y_1}(z) + \gamma_{y_2}(z) = \tilde{k}_A(t) - u_A \\ \dot{z}_2 &= \tilde{k}_B(t) - \frac{\delta_b}{\delta_a}(z_2 - \gamma_{y_1}(z)) = \tilde{k}_B(t) - \frac{\delta_b}{\delta_a} u_B. \end{aligned} \quad (12)$$

Letting  $g_1(u_A, t) := \tilde{k}_A(t) - u_A$  and  $g_2(u_B, t) := \tilde{k}_B(t) - \frac{\delta_b}{\delta_a} u_B$ , which are the input fluxes provided by the upstream source module, equation (11) yields  $\dot{u}_A = \frac{g_1(u_A, t)}{1 + \frac{d\gamma_{y_1}(z)}{du_A} + \frac{d\gamma_{y_2}(z)}{du_A}}$  and  $\dot{u}_B = \frac{g_2(u_B, t)}{1 + \frac{d\gamma_{y_1}(z)}{du_B}}$ . By comparison the latter equations with the general form given in (3a), we derive that the retroactivity to the first and to the second input can be attenuated by

minimising the quantities  $\frac{d\gamma_{y_1}(z)}{du_A} + \frac{d\gamma_{y_2}(z)}{du_A}$  and  $\frac{d\gamma_{y_1}(z)}{du_B}$ , respectively.

## V. CONCLUSIONS

In this paper we have presented a minimal CRN module that can be used to realise a molecular device for the subtraction of two molecular fluxes; this system represents a key component for the implementation of an molecular embedded feedback control system. The main contribution of this work consists in an investigation of the retroactivity properties of the proposed subtractor device. To perform this study, we have performed a singular perturbation analysis of the proposed system, assuming an interconnection with a generic upstream source module and with a downstream transcriptional module. Both the theoretical analysis and the results obtained via numerical simulation show that, time-scale separation of the connected both can attenuate the retroactivity to the output effects. These results represent a useful step towards the construction of methodologically sound approaches to the design of synthetic biological feedback controllers.

## ACKNOWLEDGMENT

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