Preprints of the
6th IFAC Conference on Foundations of Systems Biology in Engineering
The International Federation of Automatic Control
Magdeburg, Germany, October 9-12, 2016

Zero-Retroactivity Subtraction Module for Embedded Feedback Control of Chemical Reaction Networks

Mariaconcetta Bilotta [*] [1] **Carlo Cosentino** [*] [1] **Alessio Merola** [*]
Declan G. Bates [**] **Francesco Amato** [*]

[*] *Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi Magna Græcia di Catanzaro, 88100 Catanzaro, IT (e-mail: carlo.cosentino@unicz.it).*
[**] *School of Engineering, University of Warwick, Coventry, CV4 7AL, UK*

Abstract: The control of biochemical processes is a major goal in systems and synthetic biology. Current approaches are based on ad-hoc designs, whereas a general and modular framework would be highly desirable, in order to exploit the well-assessed methods of control theory. 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 behavior they exhibit when disconnected from each other. In the present work an implementation of a zero-retroactivity Chemical Reaction Network Subtractor (CRNS) is proposed and its effectiveness is investigated through singular perturbation analysis. The proposed CRNS represents a first step towards the development of a modular framework for the design of CRN-based embedded feedback control systems.

Keywords: Chemical reaction networks, retroactivity, Systems biology, synthetic biology.

1. INTRODUCTION

The development of a general control theory for biomolecular processes would require the realization of a set of basic molecular circuits that can be assembled in a modular way. A common approach for designing and analyzing a complex system is to decompose it into smaller modules, whose functions are well isolated by those of the neighboring modules. This approach has been employed for long time in engineering disciplines, such as electrical engineering and computer science and, more recently, it has been proposed also for the analysis of bio-molecular systems. Guaranteeing that the properties of individual components do not change upon interconnection is the central characteristic on the basis of modularity. Unfortunately, in biological systems modularity is generally compromised by *retroactivity*, which plays a role similar to impedance in electrical circuits, and consists in the effect of the reciprocal interactions arising from the interconnection of two modules (Del Vecchio et al. (2008); Del Vecchio (2013, 2015)).

From a design point of view, the retroactivity must be taken into account when engineering bio-molecular circuits and that suitable insulation mechanisms should be designed in order to buffer connected components from each other (Del Vecchio (2013)). Some solutions to attenuate retroactivity, based on high-gain feedback and time scale separation, are now available (Jayanthi and Del Vecchio (2011); Mishra et al. (2014)).Designs of insulation devices have been proposed in literature with the aim of attenuating retroactivity effects (Del Vecchio et al. (2008); Del Vecchio and Sontag (2009); Del Vecchio (2015)). The need for understanding the extent of modularity and attenuating the retroactivity in bio-molecular systems has become particularly pressing when designing synthetic circuits. Towards the realisation of modular embedded feedback controllers in synthetic biological systems, the availability of a well-characterised subtraction module is a key step. In the classical one-degree-of-freedom control scheme, a subtraction module is required to compare the desired set-point with the actual output of the process to be controlled. The realization of an embedded subtractor module remains, to the best of our knowledge, an open issue, as also discussed in Dolan et al. (2012). Oishi and Klavins (2011) have also addressed this problem in, though their approach requires some conditions not easy to meet in practice, whereas Chen et al. (2014) have shown that CRNs can be used to compute continuous piecewise linear functions. Cosentino et al. (2016) have investigated the general properties of a minimal CRN-based molecular subtractor and proposed some realization structures. A preliminar study of the retroactivity of these alternative structures has been conducted in Bilotta et al. (2015).

The present work proposes a zero-retroactivity CRN-based subtraction module, which can be used as a basic component for designing feedback controllers for biochemical reaction networks. It is assumed that the CRNS takes as first input the set-point flux and as second input the output flux of the controller process. In this case, it is important to minimize the retroactivity of the subtractor on the second input, in order to avoid undesired influence

[1] Equal contribution.

on the dynamics of the controlled output species. Otherwise, the subtractor would consume the output species of the controlled process, thus affecting the capability of the control system to track the desired set-point.

The paper is organized as follows: in Section 2 we discuss the general properties required from a subtraction block and translate these into two minimal CRN-based subtraction modules. Moreover, the concept of retroactivity and a general modeling scheme for the connection of bio-molecular systems are briefly recalled. In Section 3 a singular perturbation analysis of the two interconnected subtractors is carried out upon interconnection with an upstream and a downstream module. Some illustrative numerical examples are given in Section 4. Finally, Section 5, provides some concluding remarks.

2. PRELIMINARIES

2.1 Minimal properties of a molecular subtractor

Let consider a reactor, containing a generic CRN $\mathcal{C}$ comprising n species. Assume it is possible to inject from outside the reactor only species A and B, and denote with $u_A(t)$ and $u_B(t)$ the number of molecules per unit volume injected per unit time, and with $y_C(t)$ the corresponding number of molecules per unit volume of a species C produced by the CRN in the same time interval. To fix ideas, assume that $u_A(t) > u_B(t) > 0$. Furthermore, denote by

$u_A^{\mathbf{notC}}$**:** the number of molecules of A per unit volume that the CRN *irreversibly* converts into species other than C (including the null species, i.e. degradation of A) over a unit time interval;

$u_A^{\mathbf{C}}$**:** the number of molecules of A per unit volume that the CRN *irreversibly* converts into molecules of C over the same time interval (through any number of intermediate reactions).

Proposition 1. (Cosentino et al. (2016)) Assume that all the reactions in the CRN $\mathcal{C}$ exhibit unitary stoichiometric coefficients, that the input fluxes u_A, u_B are constant and that the following conditions are satisfied

$$u_A^{\mathrm{notC}} = u_\mathrm{B}, \tag{1a}$$

$$u_A^{\mathrm{C}} = u_\mathrm{A} - u_\mathrm{A}^{\mathrm{notC}}\,. \tag{1b}$$

$$u_\mathrm{A} = 0 \Rightarrow \lim_{t\to\infty} y_\mathrm{C}(t) = 0\,. \tag{1c}$$

Then, the output flux y_C tends asymptotically to the difference of the input fluxes, $u_\mathrm{A} - u_\mathrm{B}$.

Proof Conditions (1a)-(1b) yield $u_\mathrm{A}^\mathrm{C} = u_\mathrm{A} - u_\mathrm{B}$. Condition (1c) implies that C is a product of either A or a species produced from A, through an arbitrary number of intermediate reactions, and cannot be produced from other sources in the absence of A. Since the reactions have unitary stoichiometric coefficients and the conversion of A to C is irreversible, $y_\mathrm{C}(t)$ will tend asymptotically to u_A^C. Note that there is an infinite number of CRNs that satisfy conditions (1), since the conversion of A into other molecules (either C or non-C) can occur through any sequence of reactions, involving any number of species.

In order to achieve a minimal realization, only CRNs comprising just the three molecular species associated to the interconnection fluxes are considered. Under this constraints, a possible realization of the CRNS, satisfying conditions (1) is

$$\varnothing \xrightarrow{u_A} \mathrm{A} \xrightarrow{k_1} \mathrm{C} \tag{2a}$$

$$\varnothing \xrightarrow{u_B} \mathrm{B} \tag{2b}$$

$$\mathrm{A}+\mathrm{B} \xrightarrow{k_2} \circledast\,, \tag{2c}$$

where $y_\mathrm{C} = k_1 a$, the symbol "$\circledast$" means that the product of reaction (2c) can be any complex of species different from A, B and C (including the null species $\varnothing$ in the case of degradation), since it does not affect the behavior of the CRN.

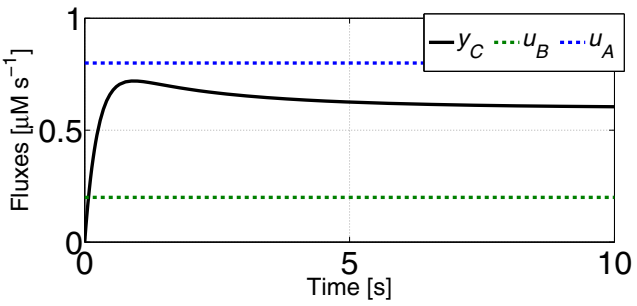

Fig. 1. Response of the isolated CRN subtraction module: The first input flux (species A) is $u_A = 0.8$ μM s^{-1}, the second input flux (species B) is $u_B = 0.2$ μM s^{-1}. The kinetic parameters in CRN (3) are set to $k_1 = 4\mathrm{s}^{-1}$ and $k_2 = 3$ (μM s)$^{-1}$ therefore the output flux (solid line) of species C represents the difference between the two input fluxes (dashed lines).

2.2 Two possible CRN-based subtraction modules

To realize the subtraction operation between the fluxes of two species, A and B, the CRN-based module we propose to employ is the following CRN.

$$\begin{aligned} \varnothing &\xrightarrow{u_A} \mathrm{A} \xrightarrow{k_1} \mathrm{C} \\ \varnothing &\xrightarrow{u_B} \mathrm{B} \\ \mathrm{A}+\mathrm{B} &\xrightarrow{k_2} \mathrm{W}\,. \end{aligned} \tag{3}$$

The dynamical system describing the behavior of CRN (3) is given by the two input-single output system

$$\dot{a} = u_A - k_1\, a - k_2\, a\, b \tag{4a}$$

$$\dot{b} = u_B - k_2\, a\, b \tag{4b}$$

$$\dot{w} = k_2\, a\, b \tag{4c}$$

$$y_C = \dot{c} = k_1\, a\,, \tag{4d}$$

where italic lowercase letters, a, b, c, and w are used to denote the concentration of species A, B, C and W, respectively.

For each molecule of B that enters the CRN (3), exactly one molecule of A is converted into species W. The molecules of A that are not degraded or converted to W, are turned into molecules of C. In this way, the output flux of species y$_C$ tends asymptotically to the difference of the two inputs fluxes u_A and u_B, see Fig. 1.

Alternatively, let us consider another CRN, which involves a species B that can exist in two forms (e.g., a protein in

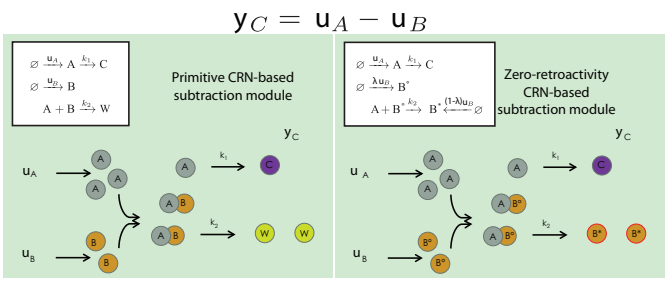

Fig. 2. Two alternative implementations of the CRNS scheme (2)

phosphorylated or dephosphorylated form), which will be denoted with B° and B*. In this case, the letter B denotes the total amount of the species (independently of the fractions of B° and B*) and with b we will denote the whole concentration of B, that is the sum of the concentrations of B° and B*, denoted by b° and b^*, respectively. We consider as input to this CRN the total flux of species B (in either one of the two forms), u_B and assume that such input flux is composed by a fraction λ of molecules of the form B°. Therefore, we have

$$\begin{aligned} u_B &= u_{B^\circ} + u_{B^*} \\ &= \lambda\, u_B + (1-\lambda)\, u_B \end{aligned}$$

and the CRN reads

$$\varnothing \xrightarrow{u_A} \mathrm{A} \xrightarrow{k_1} \mathrm{C} \tag{5a}$$

$$\varnothing \xrightarrow{\lambda\, u_B} \mathrm{B}^\circ \tag{5b}$$

$$\mathrm{A} + \mathrm{B}^\circ \xrightarrow{k_2} \mathrm{B}^* \xrightarrow{(1-\lambda)u_B} \varnothing\,. \tag{5c}$$

The two devised CRN subtraction modules are illustrated in Fig. 2. The dynamics of CRN (5) are described by the system

$$\dot{a} = u_A - k_1\, a - k_2\, a\, b^\circ \tag{6a}$$

$$\dot{b}^\circ = \lambda\, u_B - k_2\, a\, b^\circ \tag{6b}$$

$$\dot{b}^* = k_2\, a\, b^\circ + (1-\lambda)\, u_B \tag{6c}$$

$$y_C = \dot{c} = k_1\, a\,. \tag{6d}$$

2.3 Mathematical model for interconnected systems

Exploiting the framework devised in Jayanthi and Del Vecchio (2011), let us consider a generic system S with $\sigma \in D_u \subset \mathbb{R}^q_+$, $x \in D_x \subset \mathbb{R}^n_+$ and $\nu \in D_\nu \subset \mathbb{R}^p_+$ denoting concentrations of chemical species, such as proteins, enzymes, DNA sites, etc. Our aim is to investigate whether and to what extent the dynamics of system S change upon interconnection with other modules. Therefore, we consider the case in which system S is connected to an upstream and a downstream module, as illustrated in Fig.3. Looking and the interconnections scheme, we can distinguish four interconnection fluxes, denoted with f, g, r, and s. In particular, $g(\sigma, t)$ and $f(x, \sigma)$ are reaction rate vectors representing the input and output fluxes of system S. In the interconnected scheme, $g(\sigma, t)$ represents the input molecular fluxes generated from the upstream module (the *source*), whereas $f(x, \sigma)$ represents the molecular fluxes produced by system S and fed to the load module.

The reaction rates terms $r(x, \sigma)$, $s(x, \nu)$, instead, denote the *retroactivity to the input* and *retroactivity to the output*, respectively. These terms arise from the interconnection of S with the source and load module and, thus, perturb the dynamics of S with respect to the isolated case.

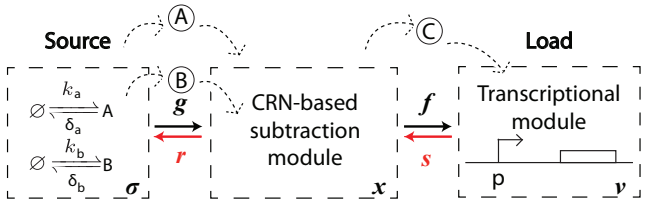

Fig. 3. The analysis of the retroactivity is realised by a modular interconnection of the CRN-based subtraction module. The CRN-based subtraction module takes as inputs the fluxes of species A and B generated from an upstream module. This source block is composed of reactions of formation and degradation for each input species. The output of the subtractor module is the flux of species C produced by the reactions of the CRNS. The transcription factor C binds to the promoter p, inducing the transcription and translation of a protein D. The red arrows denote retroactive fluxes arising upon interconnection of the CRNS with the source and load modules.

The dynamics of the interconnected system can be conveniently described by means of the following system

$$\dot{\sigma} = g(\sigma, t) + G_\alpha\, \mathcal{A}\, r(x, \sigma) \tag{7a}$$

$$\dot{x} = G_\alpha\, \mathcal{B}\, r(x, \sigma) + G_\alpha\, f(x, \sigma) + G_\beta\, \mathcal{C}\, s(x, \nu) \tag{7b}$$

$$\dot{\nu} = G_\beta\, \mathcal{D}\, s(x, \nu) + G_\beta\, l(\nu) + h(\nu, t)\,, \tag{7c}$$

where $\mathcal{A} \in \mathbb{R}^{r\times q}$, $\mathcal{B} \in \mathbb{R}^{r\times n}$, $\mathcal{C} \in \mathbb{R}^{s\times n}$ and $\mathcal{D} \in \mathbb{R}^{s\times p}$ are constant matrices, $l(\nu) \in \mathbb{R}^p$, $h(\nu, t) \in \mathbb{R}^p$ are vector fields, G_α, G_β are positive scalars that can be used to tune the relative velocity of the dynamics of the three modules. Note that a mathematical description of the unloaded system can be readily obtained by considering $s(x, \nu) = 0$ (Del Vecchio et al. (2008); Del Vecchio (2013); Mishra et al. (2014); Del Vecchio (2015)).

In the following, in place of a generic system S, we shall consider the two CRNSs (2), (5). In order to evaluate the retroactivity of the two subtractors, we shall use the CRN

$$\varnothing \underset{\delta_a}{\overset{k_a}{\rightleftharpoons}} \mathrm{A} \tag{8a}$$

$$\varnothing \underset{\delta_b}{\overset{k_b}{\rightleftharpoons}} \mathrm{B}\,, \tag{8b}$$

as source module. As for the load module, we adopt the following generic transcriptional mechanism,

$$\mathrm{C} + \mathrm{p} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{D}\,, \tag{9}$$

where p represent a promoter region, to which the species C can bind, and D denotes the complex C:p, that is the promoter with a molecule of C bound to it. Note that the total concentration of the promoter (that is the sum of the bound and unbound form) is constant over time, we will denote it by p_{TOT}.

$\bar{k}_A := \frac{max_t k_a(t)}{\delta_a}$	$\bar{k}_B := \frac{max_t k_b(t)}{\delta_b}$
$\sigma_A := \frac{a}{\bar{k}_A}$	$\sigma_B := \frac{b}{\bar{k}_A}$
$x_1 := \frac{w}{\bar{k}_A}$	$x_2 := \frac{c}{\bar{k}_A}$
$\nu := \frac{d}{\bar{k}_A}$	$\tilde{k}_A(t) := \frac{k_a(t)}{\delta_a \bar{k}_A}$
$\tilde{k}_B(t) := \frac{k_b(t)}{\delta_a \bar{k}_A}$	$\tau = \delta_a\, t$

Table 1. Change of variables for adimensionalization of system (11).

3. MAIN RESULTS

In what follows, we analyze the interconnected scheme described in the previous section, applying a singular perturbations analysis (Khalil (2002)) approach.

3.1 Singular perturbation analysis of the CRNSs

The interconnected CRN (3) corresponds to the following reaction network

$$\begin{aligned} \varnothing &\underset{\delta_a}{\overset{k_a}{\rightleftharpoons}} \mathrm{A} \xrightarrow{k_1} \mathrm{C} \\ \varnothing &\underset{\delta_b}{\overset{k_b}{\rightleftharpoons}} \mathrm{B} \\ \mathrm{A} + \mathrm{B} &\xrightarrow{k_2} \mathrm{W} \\ \mathrm{C} + \mathrm{p} &\underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{D}\,. \end{aligned} \tag{10}$$

Taking into account the conservation law $p_{\mathrm{TOT}} = p + d$, the dynamics of CRN (10) are described by the following system

$$\dot{a} = k_a(t) - \delta_a\, a - k_1\, a - k_2\, a\, b \tag{11a}$$
$$\dot{b} = k_b(t) - \delta_b\, b - k_2\, a\, b \tag{11b}$$
$$\dot{w} = k_2\, a\, b \tag{11c}$$
$$\dot{c} = k_1\, a + k_{\mathrm{off}}\, d - k_{\mathrm{on}}(p_{\mathrm{TOT}} - d)\, c \tag{11d}$$
$$\dot{d} = -k_{\mathrm{off}}\, d + k_{\mathrm{on}}(p_{\mathrm{TOT}} - d)\, c\,. \tag{11e}$$

In order to derive non-dimensional variables, let us apply the change of variables defined in Table (1).

Note that $\dot{x}_i$ will denote $\frac{dx_i}{d\tau}$). Moreover, let us define the timescale parameters, $G_1 := \frac{k_1}{\delta_a}$, $G_2 := \frac{k_2 \bar{k}_A}{\delta_a}$ and $G_3 := \frac{k_{\mathrm{off}}}{\delta_a}$, which weigh the relative velocity of the dynamics of the source, subtractor and load module, respectively. Using the adimensional variables, system (11) can then be reformulated as

$$\dot{\sigma}_A = \tilde{k}_A(t) - \sigma_A - G_1\, \sigma_A - G_2\, \sigma_A\, \sigma_B \tag{12a}$$
$$\dot{\sigma}_B = \tilde{k}_B(t) - \frac{\delta_b}{\delta_a}\, \sigma_B - G_2\, \sigma_A\, \sigma_B \tag{12b}$$
$$\dot{x_1} = G_2\, \sigma_A\, \sigma_B \tag{12c}$$
$$\dot{x_2} = G_1\, \sigma_A + G_3(\nu - \frac{x_2}{k_{\mathrm{D}}}\,(p_{\mathrm{TOT}} - \bar{k}_A\, \nu)) \tag{12d}$$
$$\dot{\nu} = -G_3(\nu - \frac{x_2}{k_{\mathrm{D}}}\,(p_{\mathrm{TOT}} - \bar{k}_A\, \nu))\,, \tag{12e}$$

where $k_{\mathrm{D}} := \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}}$ is the dissociation constant. Comparing the generic model (7) with system (12) we can identify the dynamics of the source module, that are $g_{\mathrm{A}}(\sigma_{\mathrm{A}}, t) = \tilde{k}_A(t) - \sigma_A$ and $g_{\mathrm{B}}(\sigma_{\mathrm{B}}, t) = k_B(t) - \frac{\delta_b}{\delta_a}\, \sigma_B$. This means that, when the source module is not connected to the subtractor, the dynamics of the input species are given by $\dot{\sigma}_{\mathrm{A}} = g_{\mathrm{A}}(\sigma_{\mathrm{A}}, t)$ and $\dot{\sigma}_{\mathrm{B}} = g_{\mathrm{B}}(\sigma_{\mathrm{B}}, t)$. Our goal is to evaluate how these dynamics are modified by the interconnection scheme, that is to quantify the retroactivity to the inputs of the subtractor.

In order to exploit singular perturbation analysis, we have to assume that the system dynamics can be separated into a slow and a fast subset, which is true if $G_3 \gg G_2 \gg G_1 \gg 1$. Under the latter assumption, the slow and fast subsystem decomposition can be explicated by applying the change of variables $z_1 = \sigma_A + x_1 + x_2 + \nu$, $z_2 = \sigma_B + x_1$, $y_1 = x_1$ and $y_2 = x_2 + \nu$, which yields the standard singular perturbation form

$$\dot{z}_1 = \tilde{k}_A(t) - z_1 + y_1 + y_2 \tag{13a}$$
$$\dot{z}_2 = \tilde{k}_B(t) - \frac{\delta_b}{\delta_a}(z_2 - y_1) \tag{13b}$$
$$\epsilon_1 \dot{y}_1 = (z_1 - y_1 - y_2)\,(z_2 - y_1) \tag{13c}$$
$$\epsilon_2 \dot{y}_2 = z_1 - y_1 - y_2 \tag{13d}$$
$$\epsilon_3 \dot{\nu} = -(\nu - \frac{y_2 - \nu}{k_{\mathrm{D}}}\,(p_{\mathrm{TOT}} - \bar{k}_A\, \nu))\,, \tag{13e}$$

where $\epsilon_1 := 1/G_1$, $\epsilon_2 := 1/G_2$, $\epsilon_3 := 1/G_3$.

3.2 Quantification of the retroactivity

Since the singular parameters $\epsilon_i \ll 1$, $i = 1, \ldots, 3$, the left-hand side of Eqs. (13c)-(13e) can be approximated to zero, yielding the system of algebraic equations

$$0 = (z_1 - y_1 - y_2)\,(z_2 - y_1) \tag{14a}$$
$$0 = z_1 - y_1 - y_2 \tag{14b}$$
$$0 = -(\nu - \frac{y_2 - \nu}{k_{\mathrm{D}}}\,(p_{\mathrm{TOT}} - \bar{k}_A\, \nu)) \tag{14c}$$

Eqs. (14) enables us to compute y_1, y_2 and ν as functions of $z = (z_1\ z_2)^T$, that is we can express the trajectories of the fast subsystem as instantaneous functions of the trajectories of the slow one. Let us denote by $y_1 = \gamma_{y_1}(z)$ and $y_2 = \gamma_{y_2}(z)$ the solutions of (14). By substituting γ_{y_i} for y_i in (13a)-(13b), we obtain the reduced-order system

$$\begin{aligned} \dot{z}_1 &= \tilde{k}_{\mathrm{A}}(t) - z_1 + \gamma_{y_1}(z) + \gamma_{y_2}(z) \\ &= \tilde{k}_{\mathrm{A}}(t) - \sigma_{\mathrm{A}} \\ \dot{z}_2 &= \tilde{k}_B(t) - \frac{\delta_b}{\delta_a}(z_2 - \gamma_{y_1}(z)) \\ &= \tilde{k}_B(t) - \frac{\delta_b}{\delta_a}\sigma_{\mathrm{B}}\,, \end{aligned} \tag{15}$$

whose dynamics approximate the dynamics of system (13).

At this point we can compute the modified dynamics of σ_{A} and σ_{B}: according to the change of variables defined above, $\dot{\sigma}_{\mathrm{A}} = \dot{z}_1 - \dot{\gamma}_{y_1} - \dot{\gamma}_{y_2}$ and $\dot{\sigma}_{\mathrm{B}} = \dot{z}_2 - \dot{\gamma}_{y_1}$, hence, from (15) we get

$$\begin{aligned} \dot{\sigma}_{\mathrm{A}} &= g_{\mathrm{A}}(\sigma_{\mathrm{A}}, t) - \dot{\gamma}_{y_1} - \dot{\gamma}_{y_2} \\ \dot{\sigma}_{\mathrm{B}} &= g_{\mathrm{B}}(\sigma_{\mathrm{B}}, t) - \dot{\gamma}_{y_1} \end{aligned}$$

Finally, noting that $\frac{d\gamma_y}{dt} = \frac{d\gamma_y}{d\sigma}\frac{d\sigma}{dt}$, we get

$$\begin{aligned} \dot{\sigma}_A &= \frac{g_{\mathrm{A}}(\sigma_A, t)}{1 + \mathcal{R}_A} \\ \dot{\sigma}_B &= \frac{g_{\mathrm{B}}(\sigma_B, t)}{1 + \mathcal{R}_B}\,, \end{aligned} \tag{17}$$

where $\mathcal{R}_A = \frac{\mathrm{d}\gamma_{y_1}}{\mathrm{d}\sigma_A} + \frac{\mathrm{d}\gamma_{y_2}}{\mathrm{d}\sigma_A}$ and $\mathcal{R}_B = \frac{\mathrm{d}\gamma_{y_1}}{\mathrm{d}\sigma_B}$ represent a measure of the retroactivity to the first and to the second

input, respectively. Note that, to enhance modularity of the interconnection scheme, it would be desirable to minimize $\mathcal{R}_{\rm A}$ and $\mathcal{R}_{\rm B}$, such that the subtractor exhibit low retroactivity to the inputs.

3.3 A zero-retroactivity CRNS

A possible application of the CRNS is to exploit it in the design of a modular control systems for chemical reaction networks. Employing a classical feedback control scheme, the second input of the subtractor (input species B) coincides with the controlled output of the process. Therefore, it is highly desirable to minimize the retroactivity to this input.

In this section we show that the CRN subtraction module (5) exhibits zero-retroactivity to the input species B. To this aim, we repeat the analysis performed in Sections 3.1-3.2, interconnecting (5) with the source and load modules, which yields

$$\begin{aligned} \varnothing &\underset{\delta_a}{\overset{k_a}{\rightleftharpoons}} \mathrm{A} \xrightarrow{k_{s_1}} \mathrm{C} \\ \varnothing &\underset{\delta_b}{\overset{k_b}{\rightleftharpoons}} \mathrm{B}^\circ \\ \mathrm{A} + \mathrm{B}^\circ &\xrightarrow{k_{s_2}} \mathrm{B}^* \\ \mathrm{C} + \mathrm{p} &\underset{k_{\rm off}}{\overset{k_{\rm on}}{\rightleftharpoons}} \mathrm{D}\,. \end{aligned} \tag{18}$$

Note that the source can produce only the form B°, that is we are in the case $\lambda = 1$. The model of this CRN can be readily obtained from (11) by substituting b° for b and b^* for w. Subsequently, we can exploit the same machinery used in Sections 3.1-3.2, interconnecting (5) to derive the adimensional system and the singular perturbations form. Differently from CRNS (3), in this case the dynamics of the input species B are given by the sum of the dynamics of B° and B^*, that is $\dot\sigma_{\rm B} = \dot\sigma_{\rm B^\circ} + \dot x_1$. Therefore, the analysis of the reduced-order system, obtained through the singular perturbation analysis, yields

$$\dot\sigma_{\rm B} = \dot\sigma_{\rm B^\circ} + \dot\gamma_{y_1} = g_{\rm B}(\sigma_{\rm B}, t)\,,$$

which means that the dynamycs of the input species B are not modified upon interconnection of the source and load modules with CRNS (5).

4. NUMERICAL EXAMPLES

In order to further investigate the behavior of the CRNS (3) and (5), we have performed some numerical simulations. The CRNS have been simulated in the interconnected configuration depicted in Fig. 3; therefore, the whole simulation model is provided by system (11). As mentioned in the previous section, the model of the interconnected scheme with the zero-retroactivity CRNS is identical to that in Eq. (11), once we have replaced b with b° and w with b^*. The parameters, for both models, have been set as given in Table 2.

The results of the simulations, given in Figs. 4-5, show that the dynamics of the two subtractors are identical for what concerns the output: both CRNS produce a flux of species C that converges to the ideal difference of the input fluxes generated by the source module. The behavior of the two CRNS, instead, differs in the dynamics of the second input

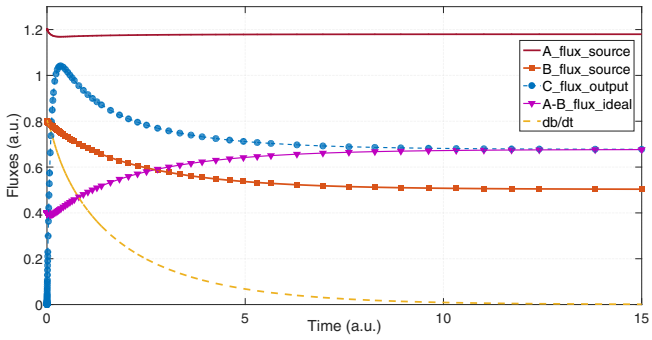

Fig. 4. Time-course of the fluxes of CRNS (3): "A flux source" and "B flux source" are the fluxes of species A and B generated by the source module; "A-B flux ideal" is the difference of the previous quantities (i.e., the output of an ideal subtractor); "C flux output" is the flux of species C produced by the subtractor (i.e., the difference actually computed by the subtractor); "dB/dt" is the dynamics of species B, as determined by the whole interconnected system.

(flux of species B). The effect of the non-zero retroactivity of CRNS (3) is clearly visible in Fig. 4: while the flux of species B produced by the source module converges to a fixed nonzero value, the value of db/dt converges to zero, thus the concentration of species B in the interconnected system reaches a steady-state. The reason is that in this subtraction module B is consumed (or transformed to other species) at a rate that eventually equals the input flux.

Conversely, in CRNS (5) the species B does not get consumed, but is rather transformed between the two forms B° and B^*; therefore, the whole dynamics of B (that is $db/dt = db^\circ/dt + db^*/dt$) coincides with the flux produced by the source module, as shown in Fig. 5.

5. CONCLUSIONS

One of the main goals of synthetic biology is the design of modular control systems for biochemical processes. A major obstacle toward this aim is represented by retroactivity effects arising in interconnected reaction networks. We have devised a CRN module that can be used as a subtractor in classical feedback control schemes and its retroactivity properties have been investigated. Using singular perturbations analysis, we have shown that a special version of such module exhibits zero-retroactivity to one of the inputs. The zero retroactivity results from the exploitation on the second input of a species B that can assume two forms (e.g., with or without phosphorylation): only one of the two forms can bind the other input species A, that enabling to count the difference in the number of molecules of A and B that are processed by the CRN per

param.	value	param.	value
k_a	1.2	δ_a	0.3
k_b	0.8	δ_b	0.2
k_1	10	k_2	5
$k_{\rm on}$	300	$k_{\rm off}$	100

Table 2. Parameter values of model (11) used in the numerical examples (arbitrary units).

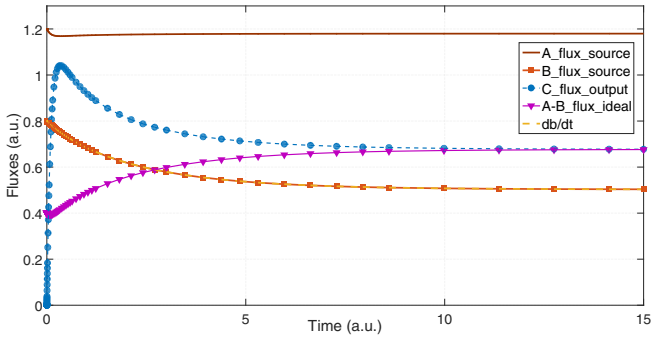

Fig. 5. Time-course of the fluxes of CRNS (5): "A flux source", "C flux source", "A-B flux ideal" are the same as in Fig. 4. Here, instead, "dB/dt" denotes the total dynamics of B, that is the sum of the dynamics of the two forms B° and B*. Analogously, "B flux source" is the total flux of B generated by the source.

unit time. The contribution of the paper is two-fold: on a first side, the devised CRNS can be used as a starting scheme to build a wet-lab implementation of a biomolecular subtractor module, using the tools of synthetic biology. On the other side, the study presented here provides new insights about the role of covalent modifications of proteins in the control of biochemical processes. By analogy with out CRNS module, indeed, we can surmise that covalent modifications are needed in physiological molecular feedback control schemes, to implement comparison-by-subtraction modules with zero-retroactivity, i.e., modules that do not consume the signaling/regulator proteins while elaborating the associated information signals.
Future work will be devoted to the study of retroactivity properties of other modules involved in control schemes and to the wet-lab implementation of the devised CRNS.

ACKNOWLEDGEMENTS

The present work is co-funded with the support of European Commission, European Social Fund and "Regione Calabria" Department 11.

REFERENCES

Bilotta, M., Cosentino, C., Bates, D.G., Salerno, L., and Amato, F. (2015). Retroactivity analysis of a chemical reaction network module for the subtraction of molecular fluxes. *Engineering in Medicine and Biology Society (EMBC), IEEE 37th Annual International Conference on*, 941–944.

Chen, H.L., Doty, D., and Soloveichik, D. (2014). Deterministic function computation with chemical reaction networks. *Natural computing*, 13(4), 517–534.

Cosentino, C., Ambrosino, R., Ariola, M., Bilotta, M., Pironti, A., and Amato, F. (2016). On the realization of an embedded subtractor module for the control of chemical reaction networks. *IEEE Transactions on Automatic Control*. doi:10.1109/TAC.2016.2523679. In press.

Del Vecchio, D. (2013). A control theoretic framework for modular analysis and design of biomolecular networks. *Annual Reviews in Control*, 37(2), 333–345.

Del Vecchio, D. (2015). Modularity, context-dependence, and insulation in engineered biological circuits. *Trends in biotechnology*, 33(2), 111–119.

Del Vecchio, D., Ninfa, A.J., and Sontag, E.D. (2008). Modular cell biology: retroactivity and insulation. *Molecular systems biology*, 4(1).

Del Vecchio, D. and Sontag, E.D. (2009). Engineering principles in bio-molecular systems: from retroactivity to modularity. In *Control Conference (ECC), 2009 European*, 658–664. IEEE.

Dolan, J., Anderson, J., and Papachristodoulou, A. (2012). A loop shaping approach for designing biological circuits. *Decision and Control (CDC), IEEE 51st Annual Conference on*, 3614–3619.

Jayanthi, S. and Del Vecchio, D. (2011). Retroactivity attenuation in bio-molecular systems based on timescale separation. *Automatic Control, IEEE Transactions on*, 56(4), 748–761.

Khalil, H.K. (2002). *Nonlinear systems*. Prentice hall, New Jersey.

Mishra, D., Rivera, P.M., Lin, A., Del Vecchio, D., and Weiss, R. (2014). A load driver device for engineering modularity in biological networks. *Nature biotechnology*, 32(12), 1268–1275.

Oishi, K. and Klavins, E. (2011). Biomolecular implementation of linear i/o systems. *Systems Biology, IET*, 5(4), 252–260.