A Synthetic Biology Approach to the Realization of Embedded Feedback Controllers for Chemical Reaction Networks

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***Abstract*— Chemical Reaction Network (CRN) models based on the mass-action law play an important role in the life sciences, since they can be used to describe dynamical processes of interest in many fields of chemistry and biology. A fundamental challenge related to this kind of systems is represented by the lack, within the framework of Systems and Synthetic Biology, of a general methodology to design control systems for CRNs. The main issue addressed by this work is the development of a general methodology for designing *embedded* feedback control schemes for an assigned CRN, i.e. controllers that are themselves realizable through a CRN. In particular, we illustrate the effectiveness of the proposed approach by designing a proportional feedback controller for a well-characterized biochemical system.**

I. Introduction

Chemical Reaction Networks (CRNs) obeying mass-action law play an important role in the context of life sciences, since they represent a convenient and concise way to model processes of interest in chemistry and biology. The seminal papers by Feinberg [1],[2] show that any CRN can be equivalently described by a set of nonlinear differential equations. In particular, a CRN whose kinetics is governed by the law of mass action can suitably model characteristic behaviours, like biological switches and cell fate decision [3], [4], [5], since it may possess a finite number of isolated equilibria [7].

Compared to other application fields, the development of controllers for CRNs is greatly hampered by the difficulty in interfacing the system with controllers based on conventional technologies and the impossibility of exploiting the well-assessed and general methods available from System and Control Theory [6]. Such considerations, have led us to the conclusion that a general methodology to realize an *embedded* CRN-based controller may be based on the realization and interconnection of some elementary building blocks made up of CRNs, such that the interfacing issues can be easily overcome.

This paper deals with the problem of designing and realizing a proportional output feedback controller for a single-input single-output (SISO) CRN. In particular, since the implementation of a classical proportional output feedback control scheme requires a) the computation of the error signal and b) the amplification of the error, a possible way to realize such modules via CRNs is discussed.

The paper is organized as follows: in Section II some essential concepts from CRN theory are reviewed. In Section III the proposed embedded feedback control scheme is discussed, and possible solutions for the realization of the needed CRN-based amplification and subtraction blocks are illustrated. Section IV presents an application example. Finally, in Section V, some concluding remarks are given.

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II. Preliminaries on CRNs

Any CRN, taken together with a specification of reaction rate functions, gives rise to a system of ordinary differential equations, usually nonlinear. The derivation of the dynamical model of a CRN is based on the law of mass action [8], that is, for an elementary reaction the rate of reaction is proportional to the product of the concentrations of the reactants.

A. Zero-Input CRNs

Consider, for example, a reactor containing three species, denoted by A_1, A_2 and A_3, and the following reaction network

$$A_1 + A_2 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} 2A_3 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} A_4\,, \tag{1}$$

where κ_i, $i = 1, \dots, 4$, are the kinetic costants.

Denoting by x_i the concentrations of the species A_i, $i = 1, \dots, 4$, respectively, under the assumption of mass-action kinetics, we have two elementary reversible reactions, which occur at rates v_i, equal to

$$v_1 = \kappa_1 x_1 x_2 - \kappa_2 x_3^2 \tag{2a}$$

$$v_2 = \kappa_3 x_3^2 - \kappa_4 x_4\,. \tag{2b}$$

The evolution over time of the species concentrations is described by a system of ordinary differential equations, which can be rewritten in compact form as

$$\dot{x} = N v(x)\,, \tag{3}$$

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where $x = \begin{pmatrix} x_1 & x_2 & x_3 & x_4 \end{pmatrix}^T$ is the species concentrations vector, $v = \begin{pmatrix} v_1 & v_2 \end{pmatrix}^T$ is the reaction rates vector and

$$N = \begin{pmatrix} -1 & 0 \\ -1 & 0 \\ 2 & -2 \\ 0 & 1 \end{pmatrix}$$

is the *stoichiometric* matrix.

In general, any CRN composed of n species and r reactions, following mass-action kinetics, can be written in the form (3), where $x \in \mathbb{R}^n$, $v \in \mathbb{R}^r$, and $N \in \mathbb{R}^{n \times r}$. For example, referring to reaction (1), $n = 4$, $r = 2$ and $N \in \mathbb{R}^{4 \times 2}$.

From a system theoretic viewpoint, system (3) is a zero-input, nonlinear finite-dimensional system. Since the functions $v(\cdot)$, at the right-hand side of (3), are polynomials in the variables x_i, system (3) is also called a nonlinear *polynomial* system.

B. Forced CRNs

Assume that the reaction network (1) is subject to an affluent flux of species A_1, say u_{A_1}; according to [1], the corresponding network diagram takes the form

$$\begin{gathered} A_1 + A_2 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} 2A_3 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} A_4 \\ \varnothing \xrightarrow{u_{A_1}} A_1 \,, \end{gathered} \tag{4}$$

where $\varnothing \xrightarrow{u_{A_1}} A_1$ is a fictitious reaction, which takes into account the affluent input flux. The presence of the input flux modifies the equation of x_1, which reads

$$\dot{x}_1 = -\kappa_1 x_1 x_2 + \kappa_2 x_3^2 + u_{A_1} \tag{5}$$

We can also define an *output flux*: assume that our goal is the regulation of the flux of A_3; therefore, letting $y_3 := \dot{x}_3$, we obtain a complete input-state-output description of network (4).

In general, a network of n species, m input fluxes and p output fluxes, is described by a system of differential and algebraic equations in the form

$$\dot{x} = Nv(x) + Fu =: f(x,u) \tag{6a}$$
$$y =: g(x,u)\,, \tag{6b}$$

where $F \in \mathbb{R}^{n \times m}$ defines the contributions of the input fluxes $u \in \mathbb{R}^m$ to the concentrations of the various species and the output fluxes vector $y \in \mathbb{R}^p$.

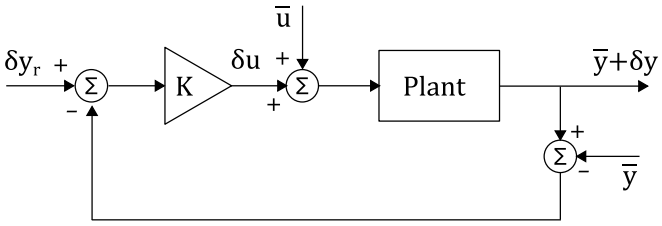

Fig. 1. Closed-loop output-feedback control scheme around an equilibrium condition.

III. Realization of an Output Feedback Controller

In this section we shall illustrate an approach to the realization of a proportional output feedback controller for a CRN, exploiting an interconnection of CRN-based modules. For the sake of simplicity we shall consider the case of a SISO CRN, so that the controller requires only scalar amplification and subtraction operations.

A. Output-feedback control around an isolated equilibrium point

Let us refer to system (6), and assume that $\overline{x} \in \mathbb{R}^n$ is an isolated equilibrium point corresponding to the constant input $\overline{u} \in \mathbb{R}^m$; furthermore, let $\overline{y}$ denote the corresponding value of the output, i.e.

$$0 = Nv(\overline{x}) + F\,\overline{u} =: f(\overline{x}, \overline{u}) \tag{7a}$$
$$\overline{y} = g(\overline{x}, \overline{u})\,. \tag{7b}$$

The study of the motion of system (6), subject to $\overline{u}$, around $\overline{x}$ can be reduced to the study of the motion around the zero equilibrium of a suitable system. Indeed, the change of variables

$$\delta x = x - \overline{x} \tag{8a}$$
$$\delta u = u - \overline{u} \tag{8b}$$
$$\delta y = y - \overline{y}\,, \tag{8c}$$

yields

$$\delta\dot{x} = \dot{x} = f(\delta x + \overline{x}, \delta u + \overline{u}) = \hat{f}(\delta x, \delta u)\,, \tag{9}$$

which satisfies $\hat{f}(0,0) = 0$. Also

$$\delta y = y - \overline{y} = g(\delta x + \overline{x}, \delta u + \overline{u}) - \overline{y} = \hat{g}(\delta x, \delta u)\,. \tag{10}$$

Assume that a proportional output feedback control law in the form

$$\delta u = K\left(\delta y_r - \delta y\right)\,, \tag{11}$$

is designed for system (9)-(10); we have

$$u = \delta u + \overline{u} = \overline{u} + K\left(\delta y_r - \delta y\right)\,. \tag{12}$$

Looking at the final control scheme, depicted in Figure 1, it is seen that the controller implementation requires the realization of the amplification block K and the subtractor block. A possible solution is proposed in the following sections.

B. Realization of a CRN-based amplification block

A possible realization of the amplification block is yielded by the following CRN

$$\begin{aligned} \varnothing \xrightarrow{u_A} A \xrightarrow{k_1} A + C \\ B + C \xrightarrow{k_2} B \\ C \xrightarrow{k_3} \varnothing \,. \end{aligned} \tag{13}$$

It is worth remarking that the given reactions do not have to be interpreted literally, but they can be a concise form to describe a more complex, though equivalent, mechanism. For instance, reaction $A \xrightarrow{k_1} A + C$ might equivalently be replaced by $A + Z \xrightarrow{k_1'} A + C$ with a constant source of species Z. In this case, the species Z would not have any influence on the other reactions; therefore, for the sake of simplicity, we can write the reaction system in a more compact form by omitting species Z. By applying the law of mass action, we can readily calculate the model describing the behaviour of CRN (13), which reads

$$\dot{a} = u_A \tag{14a}$$
$$\dot{b} = 0 \tag{14b}$$
$$\dot{c} = k_1\, a - k_2\, b\, c - k_3\, c \tag{14c}$$
$$y = \dot{c}\,, \tag{14d}$$

where italic lowercase letters, a, b and c are used to denote the concentration of species A, B and C, respectively, and y denotes the output flux.

Note that the same amount of species B is produced and consumed by the above reactions at any time, thus the concentration keeps constant, that is $b(t) = b(0) =: \bar{b}$.

The CRN (14) is a SISO linear system, where u_A and y are the input and the output fluxes respectively; we can easily analyze the input-output behaviour of such CRN by exploiting the Laplace transform, which yields

$$a(s) = \frac{1}{s}\, u_A(s) \tag{15a}$$
$$s\, c(s) = k_1\, a(s) - k_2\, \bar{b}\, c(s) - k_3\, c(s) \tag{15b}$$
$$y(s) = s\, c(s)\,. \tag{15c}$$

By substituting (15a) into (15b) and the resulting equation into (15c), we get the input-output transfer function of the amplifier block

$$\frac{y(s)}{u_a(s)} = \frac{k_1}{\left(s + k_3 + k_2 \bar{b}\right)}\,. \tag{16}$$

Equation (16) is the transfer function of a system with steady-state gain equal to $K = k_1 / \left(k_3 + k_2 \bar{b}\right)$. Note that, by varying the initial concentration of species B, the proposed amplifier scheme can be tuned to yield different amplification values.

C. Realization of a subtraction block via CRN

To realize the subtraction operation between the fluxes of two species, A and B, we propose to employ the following CRN[1]

$$\begin{aligned} \varnothing \xrightarrow{u_A} A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \\ \varnothing \xrightarrow{u_B} B \\ B + C \xrightarrow{k_3} B^*\,. \end{aligned} \tag{17}$$

The dynamical system describing the behaviour of CRN (17) is given by the two input-single output system

$$\dot{a} = k_2\, c - k_1\, a + u_A \tag{18a}$$
$$\dot{b} = -k_3\, b\, c + u_B \tag{18b}$$
$$\dot{b}^* = k_3\, b\, c \tag{18c}$$
$$\dot{c} = k_1\, a - k_2\, c - k_3\, b\, c \tag{18d}$$
$$y = \dot{c}\,, \tag{18e}$$

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

In network (17), C is produced from A in a reversible reaction, whose equilibrium depends on the ratio k_1/k_2. Then, after binding B, C is degraded and the molecule B undergoes a transformation (e.g. a phosphorylation), turning into its inactive form B*, which is no longer capable of degrading C.

It is easy to show that, when the CRN is isolated, that is $u_\mathrm{A} = u_\mathrm{B} = 0$, if reaction A $\xrightarrow{k_1}$ C is sufficiently fast, that is $k_1 >> k_2$, the concentration of C will tend to a steady-state value equal to the difference between the concentrations of A and B (the proof will not be reported here due to space limitations). Moreover, under the assumption that the rates of variation of the affluent fluxes u_A and u_B are slow with respect to the inner dynamics of CRN (17), the same property holds also for the flux u_C, which converges to the difference of the input fluxes.

[1]Here we use A, B and C to denote generic species, not the same ones used in the realization of the CRN amplifier

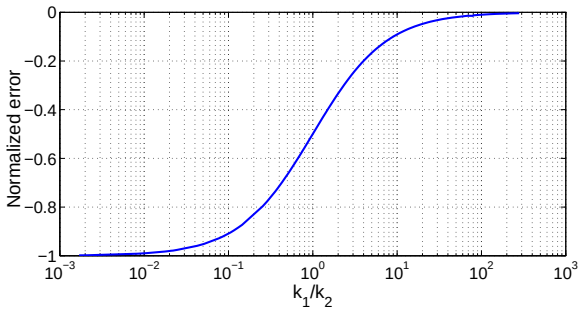

Fig. 2. Normalized error of the subtraction block: the figure summarizes the results obtained in 100 simulations using different random values for the input fluxes and the kinetic parameters.

Numerical simulations, reported in Figure 2, confirm that CRN (17) actually behaves as a subtraction module when the kinetic constants satisfy the aforementioned conditions.

IV. Case-study: control of an enzymatic reaction

This section illustrates the design and realization of a CRN-based closed-loop output-feedback control system through the interconnection of an embedded feedback controller (assembled from the CRN modules discussed above) with a CRN to be controlled.

A. Open–loop system

Let us consider a well-known CRN, namely the Michaelis-Menten irreversible enzymatic reaction mechanism

$$S + E \underset{k_r}{\overset{k_f}{\rightleftharpoons}} E : S \xrightarrow{k_{cat}} P + E \,. \tag{19a}$$

This CRN, which is a fundamental module of numerous biochemical pathways, comprises a reversible reaction for the formation of a enzyme-substrate complex E:S from the free enzyme E and the substrate S; subsequently, the bound substrate is transformed into product P and released through an irreversible reaction along with the enzyme molecule, which is then free to catalyze the transformation of other substrate molecules.

Using the law of mass action, the plant dynamics can be described through the nonlinear system

$$\dot{x}_1 = k_r\, x_2 - k_f\, x_1\, x_3 + u \tag{20a}$$

$$\dot{x}_2 = -(k_r + k_{\text{cat}})\, x_2 + k_f\, x_1\, x_3 \tag{20b}$$

$$\dot{x}_3 = (k_r + k_{\text{cat}})\, x_2 - k_f\, x_1\, x_3 \tag{20c}$$

$$\dot{x}_4 = k_{\text{cat}}\, x_2 \,, \tag{20d}$$

where x_1, x_2, x_3, x_4 denote the concentrations of substrate, complex, enzyme, product, respectively. System (20) is controlled through an input flux u affecting the substrate concentration. The objective of the control system is the regulation of the flux of P to a desired set–point y_r; therefore, the process output is chosen to be $y = \dot{x}_4$.

If the system is forced with a constant input flux $\bar{u}$, the state variables x_1, x_2, x_3 converge to the steady-state values

$$\bar{x}_1 = \frac{(k_r + k_{\text{cat}})\,\bar{u}}{k_f\, k_{\text{cat}}\, \bar{x}_3} \,,\; \bar{x}_2 = \frac{\bar{u}}{k_{\text{cat}}} \,,\; \bar{x}_3 = e_{\text{tot}} - \frac{\bar{u}}{k_{\text{cat}}} \,, \tag{21}$$

where $e_{\text{tot}} = x_2(t) + x_3(t)$ is the total concentration of enzyme (that is the free plus the bound form), which keeps constant at any time, since the enzyme is neither consumed nor produced in the enzymatic reaction. Note that equation (20d) is decoupled by the rest of the model, since species P does not influence the dynamics of the other species, whereas the flux of P depends on the concentration of complex E:S. Therefore, when fed with a constant flux of substrate, $\bar{u}$, the system will eventually return a constant flux of product, namely $\bar{y} = \bar{u}$.

For the considered CRN, the reaction rate constants are set to $k_f = 15\,(\mu M\,\mu s)^{-1}$, $k_r = 6\,\mu s^{-1}$, $k_{\text{cat}} = 0.6\,\mu s^{-1}$ and we assume $e_{\text{tot}} = 0.5\,\mu M$.

B. Controller design

The control goal is to regulate the response of the closed-loop system to the set–point $y_r = 0.2\,\mu M\,\mu s^{-1}$. Moreover, the robustness of the control performance is an important issue to be considered since the model parameters may be uncertain and affected by unmeasurable perturbations. Therefore, we will take into account both performance and robustness requirements in the design of our control system. The design will be conducted according to a classical loop-shaping approach, based on the frequency response of the linearized system. Note that, if we consider only the subsystem (20a)-(20c), under a constant input $\bar{u}$, such subsystem can be linearized in the neighborhood of the steady-state condition (21) and a nominal value of the output equal to $\bar{y}$. The following requirements on the closed-loop system are taken into account for the controller design: i) settling time at 1% of the final value of the step response $T_s < 35\,\mu s$; ii) Gain Margin $GM > 10$ dB and Phase Margin $PM > 120°$. The desired output $y = 0.2\,\mu M s^{-1}$ can be achieved by injecting into the CRN plant a feed–forward input $\bar{u} = 0.2\,\mu M\,\mu s^{-1}$, which yields the steady–state equilibrium values

$$\bar{x}_1 = 0.88 \,, \quad \bar{x}_2 = 1/3 \,, \quad \bar{x}_3 = 1/6 \,.$$

The controller gain K is tuned by analyzing the linear response of the closed–loop system both in the frequency and time domain. A settling time $T_s = 32.2\,\mu s$ and robustness

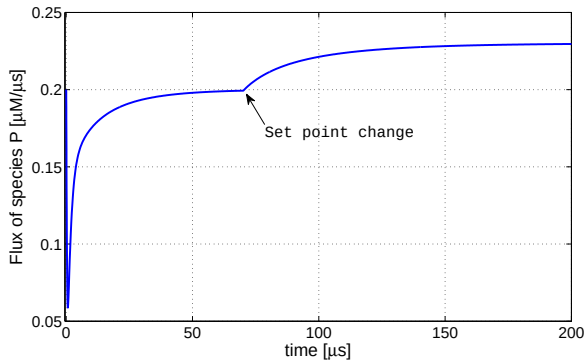

Fig. 3. System response to a step input: y_r is initially set to the nominal value $0.2\,\mu M/\mu s$; at $t = 70\,\mu s$ a step change is applied to set $y_r = 0.23\,\mu M/\mu s$.

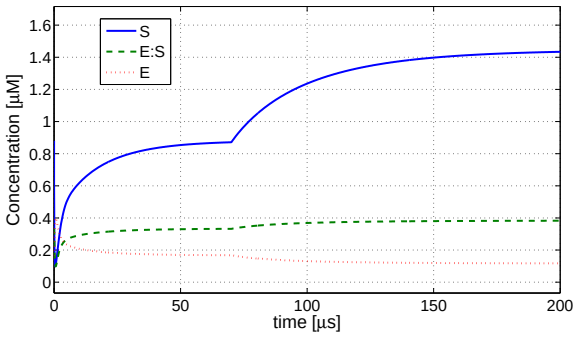

Fig. 4. Evolution of the species involved in the controlled CRN during the experiment described in Figure 3.

margins $GM = \infty$ and $PM = \infty$ can be guaranteed for $K = 0.8$.

A suitable amplifier module can be realized using the CRN (13); the parameter values are set to $k_1 = 9\,\mu s^{-1}$, $k_2 = 2\,\mu M^{-1}\,\mu s^{-1}$, $k_3 = 3\,\mu s^{-1}$. The amplifier gain can be tuned to the desired value $K = 0.8$ by assigning to species B an initial concentration $\bar{b} = 4.12\,\mu M$.

Regarding the subtraction block, it has been realized using the CRN (17). For this module, we chose the following values of the kinetic constants: $k_1 = 300\,\mu s^{-1}$, $k_2 = 2\,\mu s^{-1}$, $k_3 = 1\,\mu M^{-1}\,\mu s^{-1}$.

Finally, the interconnection of the three CRNs is implicitly realized by opportunely matching the input and output species of the modules: the output species of the CRN process, P, coincides with the second input species B in the subtraction CRN; the fist input species A in the subtraction CRN is used to specify the set-point y_r; the output species C in the subtraction CRN coincides with the input species A in the amplifier; the output species C in the amplifier CRN coincides with the input species S of the CRN process.

As shown in Figures 3 and 4, the closed-loop nonlinear control system, implemented through amplifiers and subtraction blocks, exhibits a satisfactory performance, also with moderate control effort.

V. Conclusions

In the present paper we have presented a novel approach to the problem of controlling the response of CRNs. A key feature of the proposed method is the realization of the control scheme, which is based on the assembly of submodules realized through CRNs, namely the amplification and the subtraction blocks. The proposed CRN modules have been first analyzed under isolated conditions and then assembled to realize an output-feedback proportional controller, with the aim of controlling the product flux of an enzymatic reaction. The *in silico* experiments have shown that the proposed approach is suitable and yields promising results. These results pave the way to a general theory for the design of CRN controllers, which will be the subject of future work.

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