Synthetic Biology: An Approach Based on Classical Control

UNIVERSITÀ MAGNA GRÆCIA DI CATANZARO

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Overview

Introduction

Synthetic Biology approach to the control of CRNs

- Preliminaries on Chemical Reaction Networks
- Zero-input CRN
- Forced CRN

Realization of basic modules for a CRN-based controller

- Feedback control: modular vs. embedded
- CRN-based amplifier module
- CRN-based subtraction module

Conclusions

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.

- Moreover, under suitable assumptions, the formalism of reaction networks can be adopted in many other application fields, even beyond life sciences.

- The seminal paper by Feinberg shows that any CRN can be equivalently described by a set of nonlinear differential equations.

- From a system theoretic point of view, CRNs can be expressed in state-space form, with the species concentrations representing the state variables, the incoming species fluxes into the chemical reactor being the inputs, and the outgoing fluxes being the outputs.

Feinberg M. , Chemical reaction network structure and the stability of complex isothermal reactors — I. The deficiency zero and deficiency one theorems. **Chemical Engineering Science**, 42(10):2229–2268.

Introduction

- In particular a CRN system, whose kinetics is governed by the law of mass action, may exhibit, depending on the network topology, a finite number of isolated equilibria.

- CRNs possessing multi-stable (hence isolated) equilibrium points are very appealing, both from the biological and from the control engineering perspective (see, for example, the study of the domains of attraction in a tumor model, or of the robust bi-stability of the galactose gene regulatory network).

- Therefore, we shall focus on the controller design for such kind of CRNs.

Merola A., Cosentino C., Amato F., An insight into tumor dormancy equilibrium via the analysis of its domain of attraction. **Biomedical Signal Processing and Control,** 212–219, 2008.

Cosentino C., Salerno L., Passanti A., Merola A., Bates D. G., Amato F. Structural bistability of the GAL regulatory network and characterization of its domain of attraction. **J. Computational Biology**, 19:148–162, 2012.

Introduction

- Compared to other application fields, the development of controllers for CRNs is greatly hampered by the issues arising when the system to be controlled has to be interfaced with controllers based on conventional technologies (e.g. an electronic microcontroller).

- The molecular scale of the problem, indeed, entails the lack of effective real-time sensing and actuation devices and, therefore, the impossibility of exploiting the well-assessed and general methods available from System and Control Theory.

Introduction

- Such considerations have led us to the conclusions that a general methodology for CRNs controller design requires the realization of some basic subsystems that can be assembled in a modular way in order to realize an embedded CRN-based controller.

- An important feature pertaining to such modules consists of the fact that they have to be implemented through CRNs themselves, such that the interfacing issues can be easily overcome.

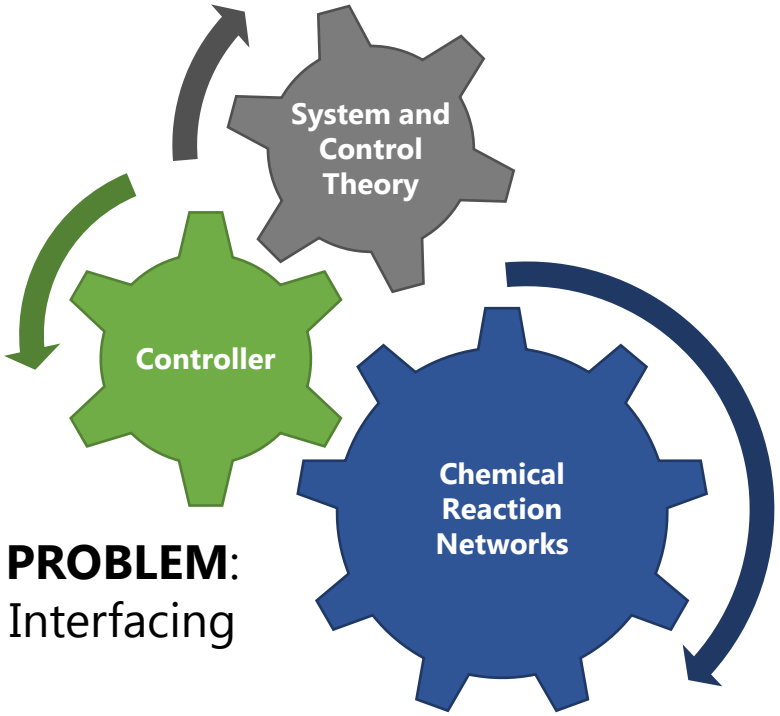
- This strategy is reminiscent of those adopted in earlier control systems, prior to the advent of electronics, when the realization technology of the control devices had to match that of the plant.

Introduction

- Starting from the above ideas, our goal is to developing a general methodology for the design of CRN-based dynamical controllers.
- The main contribution consists of the design and realization of a PI feedback controller for a single-input single-output (SISO) CRN, based on the realization and interconnection of some elementary building blocks made up of CRNs.
- In particular, since the implementation of a classical PI control scheme requires a) the computation of the error signal and b) the amplification and the integration of the error, a possible solution for the realization of such modules via CRNs is discussed.

SYNTHETIC BIOLOGY

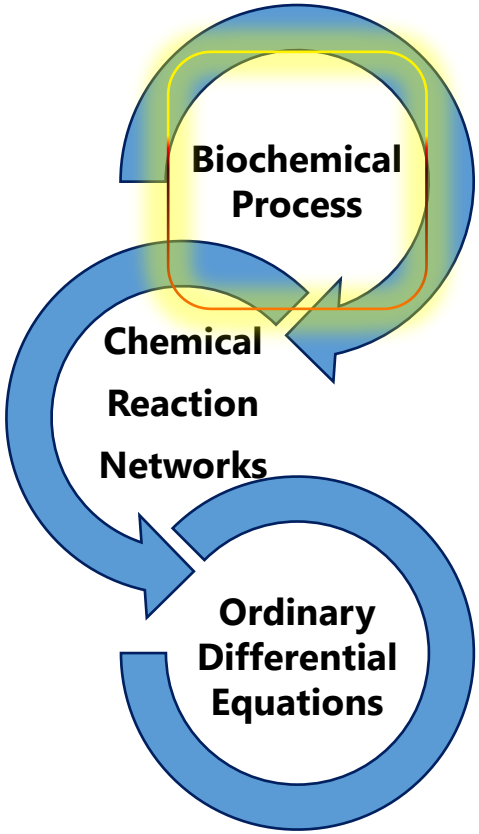
GOAL: to extend or modify the behavior of organisms and engineer them to perform new tasks.

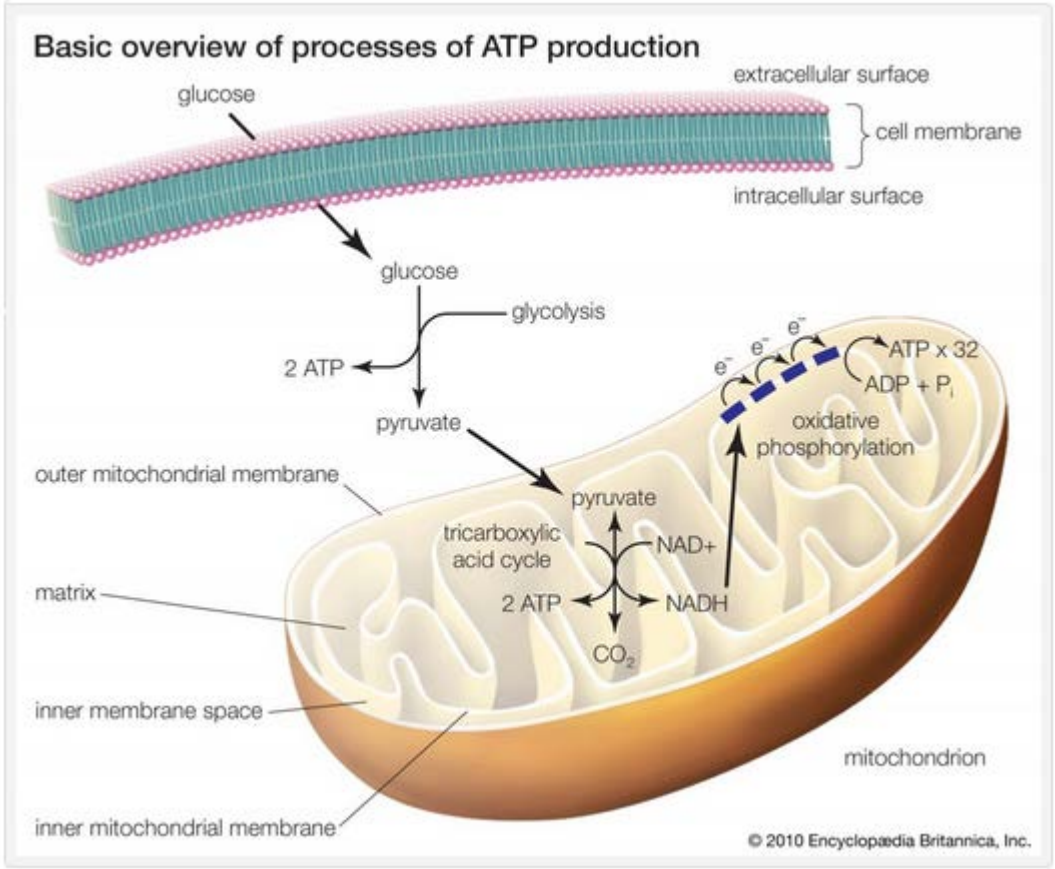


PROBLEM: Interfacing

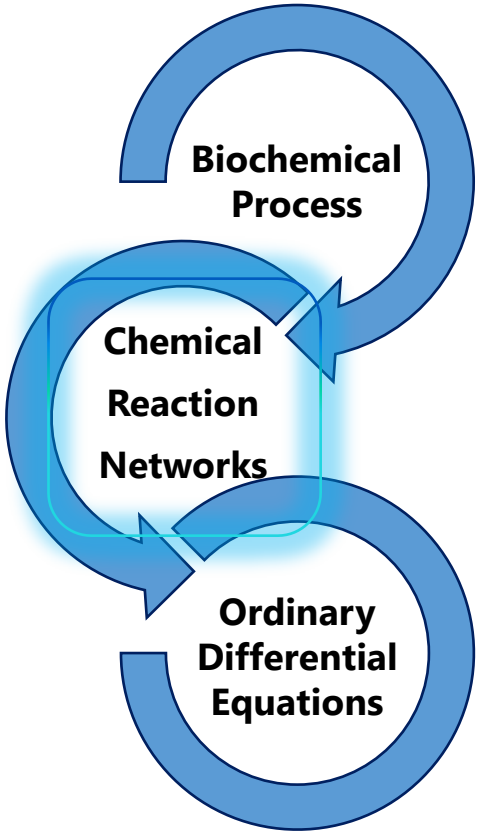
Preliminaries on Chemical Reaction Networks

- **Study of biochemical process**





- **Modeling by Chemical Reaction Networks**



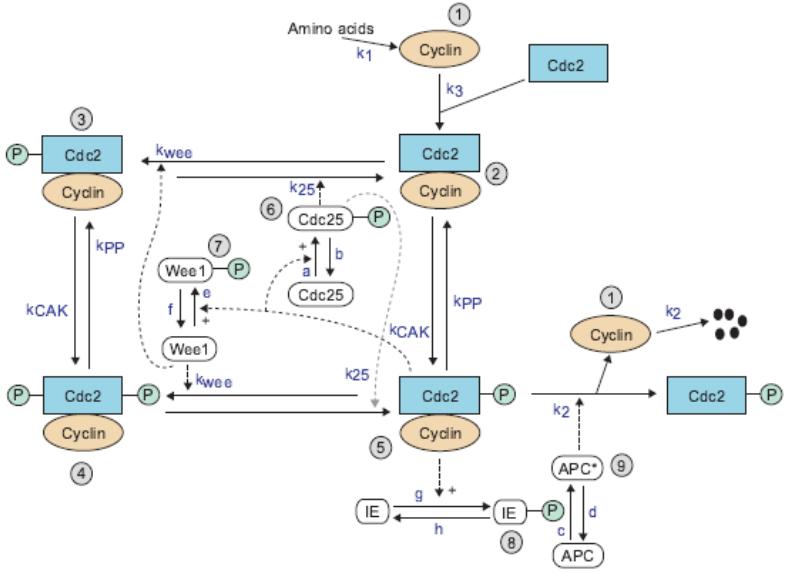
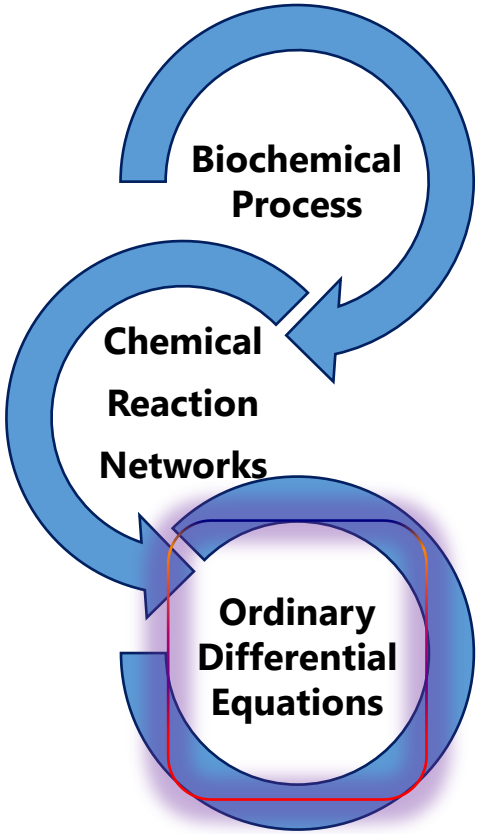


Figure 6.1: The biochemical network driving the cell cycle in *Xenopus* frog eggs. The 9 proteins interact dynamically through chemical reactions to generate periodic variations in the concentration of MPF (protein 5), which in turn controls the phase transitions of the cell cycle.

Design and Analysis of Feedback Structures in Chemical Plants and Biochemical Systems.

Henning Schmidt (2004)

- **Analysis by ODEs**



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

v_1: A+B $\rightarrow^{k_1}$ C+C Hetero
v_2: A+C $\rightarrow^{k_2}$ D Hetero
v_3: C $\rightarrow^{k_3}$ E+F Unary
v_4: F+F $\rightarrow^{k_4}$ B Homeo

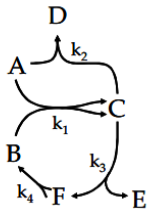
Chemical reactions

N	v_1	v_2	v_3	v_4
A	-1	-1		
B	-1			1
C	2	-1	-1	
D		1		
E			1	
F			1	-2

Stoichiometry, **N**

l
l_1	$k_1[A][B]$
l_2	$k_2[A][C]$
l_3	$k_3[C]$
l_4	$k_4[F]^2$

Rate laws, l



Flux

Modelling zero-input CRNs

Consider, for example, a reactor containing four species, denoted by A_1, A_2, A_3 and A_4, and the following reaction network

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

where $k_i, i = 1, \ldots .4$ are kinetic constants.
Denoting by x_i the concentrations of the species A_i, i = 1, . . . , 4, respectively, under the assumption of mass-action kinetics, let us consider the first reaction

$$A_1 + A_2 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} 2A_3$$

We obtain two differential equations, one for A_1 and one for A_2

$$\dot{x}_1 = -k_1 x_1 x_2 + k_2 x_3^2$$
$$\dot{x}_2 = -k_1 x_1 x_2 + k_2 x_3^2$$

Modelling zero-input CRNs

From the second reaction

$$2A_3 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} A_4$$

we obtain the third equation

$$\dot{x}_4 = -k_4 x_4 + k_3 x_3^2$$

Finally, since A_3 is involved in both reactions,

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

we get

$$\dot{x}_3 = 2k_1 x_1 x_2 - k_2 x_3^2 - k_3 x_3^2 + 2k_4 x_4$$

Modelling zero-input CRNs

Therefore we obtain a set of 4 differential equations

$$\dot{x}_1 = -k_1 x_1 x_2 + k_2 x_3^2$$
$$\dot{x}_2 = -k_1 x_1 x_2 + k_2 x_3^2$$
$$\dot{x}_3 = 2k_1 x_1 x_2 - k_2 x_3^2 - k_3 x_3^2 + 2k_4 x_4$$
$$\dot{x}_4 = -k_4 x_4 + k_3 x_3^2$$

Modelling forced CRNs

Assume that the reaction network is subject to an **affluent flux** of species A_1, say u_{A_1}

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

$$\varnothing \xrightarrow{u_{A_1}} A_1 ,$$

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 a_1, which reads

$$\dot{x}_1 = -k_1 x_1 x_2 + k_2 x_3^2 + u_{A_1}$$

IN GENERAL

A CRN comprising n species, $x \in \mathbb{R}^n$, m input fluxes, $u \in \mathbb{R}^m$, and p output fluxes, $y \in \mathbb{R}^p$, can be described by a system of differential and algebraic equations in the form

$$\dot{x} = f(x, u)$$
$$y = Cx$$

Modelling zero-input CRNs

There exists well established procedures to put any CRN in the form of set Of differential equations

Such procedures can be implemented via a computer algorithm, as well as the computation of the equilibrium points

Feinberg M. , Chemical reaction network structure and the stability of complex isothermal reactors — I. The deficiency zero and deficiency one theorems. **Chemical Engineering Science**, 42(10):2229–2268.

Output-feedback control around an isolated equilibrium point

$\bar{u} \in \mathbb{R}^m$: constant input
$\bar{x} \in \mathbb{R}^n$: isolated equilibrium point
$\bar{y}$: corresponding value of the output

$$0 = Nv(\bar{x}) + F(\bar{u}) \coloneqq f(\bar{x}, \bar{u})$$
$$\bar{y} = g(\bar{x}, \bar{u})$$

Linearization Analysis:

$$\delta x = x - \bar{x}$$
$$\delta u = u - \bar{u}$$
$$\delta y = y - \bar{y}$$

yields

$$\delta \dot{x} = \dot{x} = f(\delta x + \bar{x}, \delta u + \bar{u}) = \hat{f}(\delta x, \delta u)$$

$$\delta y = y - \bar{y} = g(\delta x + \bar{x}, \delta u + \bar{u}) - \bar{y} = \hat{g}(\delta x, \delta u)$$

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

Realization of a CRN-based controller

Control law:

$$\delta u = K(\delta y_r - \delta y)$$
$$\bar{y} = g(\bar{x}, \bar{u})$$
$$u = \delta u + \bar{u} = \bar{u} + K_P(\delta y_r - \delta y)$$

Closed-loop output feedback control scheme around an equilibrium condition

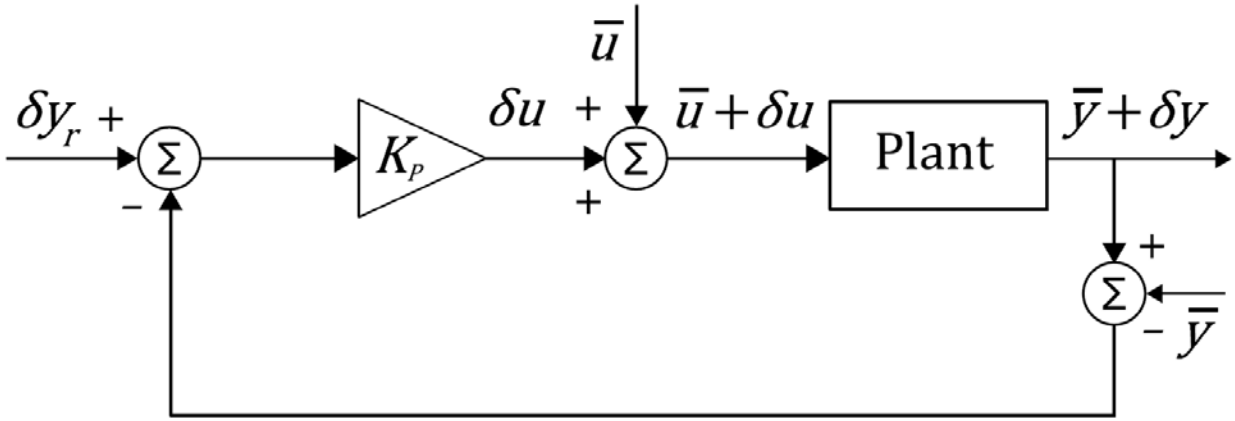
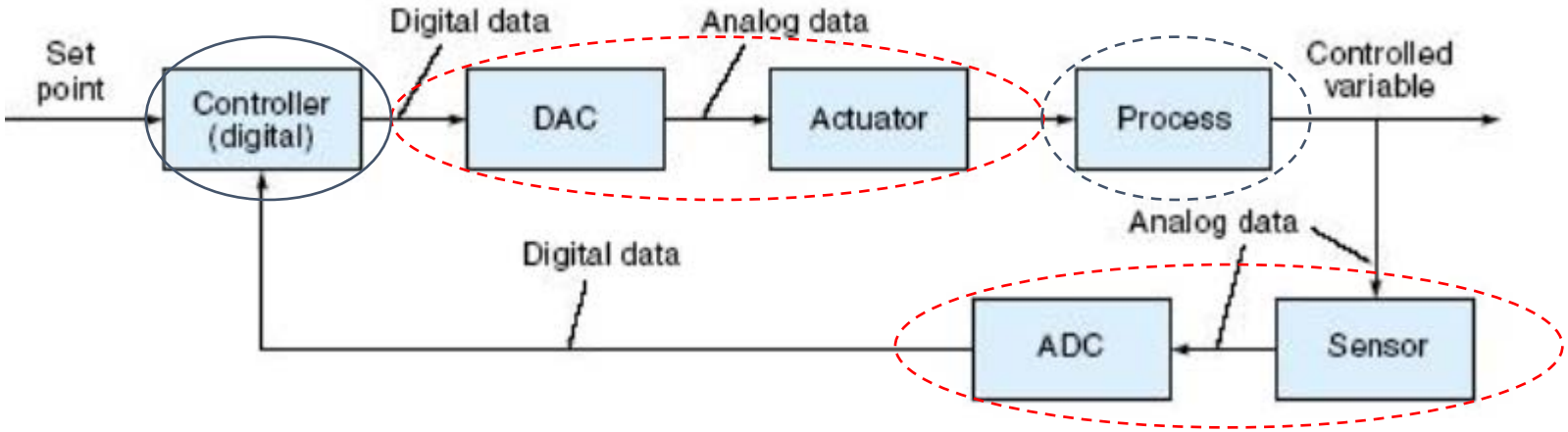


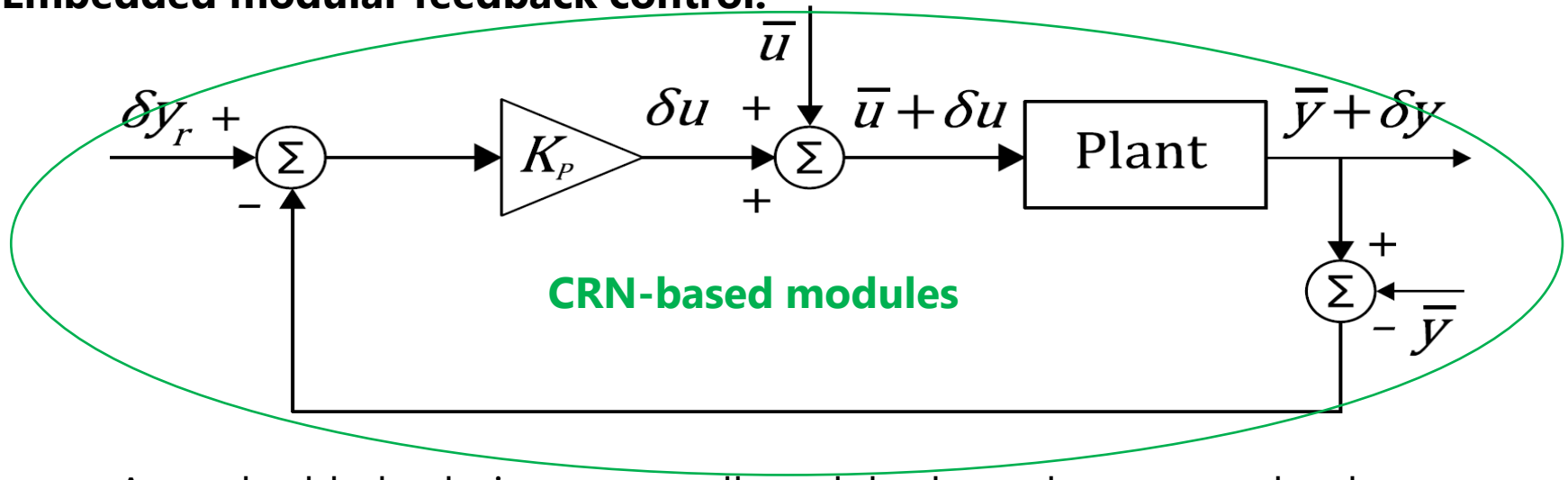
Fig. 1: Synthetic proportional feedback controller.

Embedded feedback control

Modular feedback control:



Embedded modular feedback control:

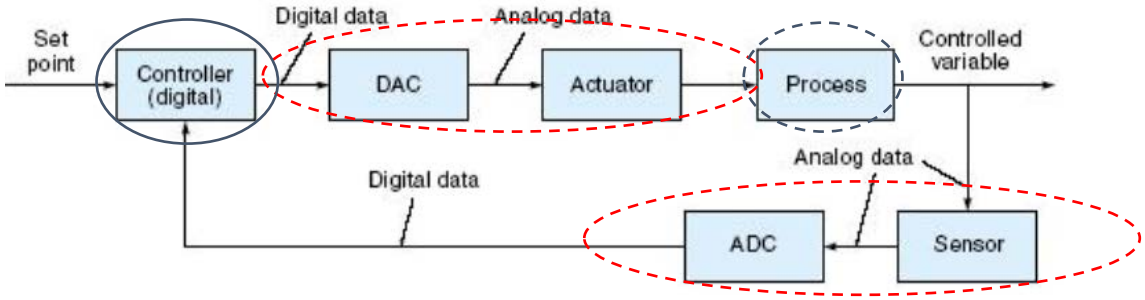


An embedded solution means all modules have the same technology

Feedback control: modular vs. embedded

Modular:

- necessity of appropriate modules to interconnect main components



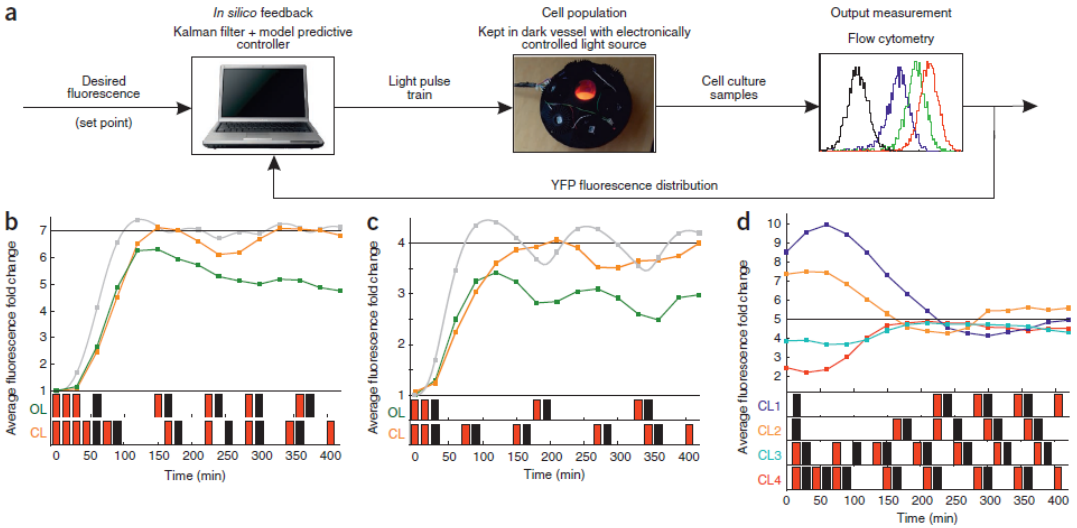


Figure 2 *In silico* feedback achieves robust regulation of gene expression fold change. (**a**) *In silico* feedback control scheme for the light-activated gene system. (**b**) Regulation of average YFP fluorescence to sevenfold over a 7-h period using *in silico* feedback (orange). A pre-computed light pulse train that achieves set point regulation when applied to the mathematical model (gray) did not achieve the desired fold induction when applied in open loop to the biological construct (green). In contrast, closed-loop feedback control achieves the desired fold induction. OL and CL denotes open- and closed-loop control, respectively. (**c**) Regulation of average YFP fluorescence to fourfold above basal over a 7-h period. Open- and closed-loop pulse trains determined as in **b**. (**d**) Regulation of average YFP to fivefold above basal over a 7-h period, starting from a randomly perturbed culture. Closed-loop control achieves the desired set point, irrespective of the initial conditions of the system.

In silico feedback for in vivo regulation of a gene expression circuit.

A. Milias-Argeitis, S. Summers, J. Stewart-Ornstein, I. Zuleta, D. Pincus, H. El-Samad, M. Khammash & J. Lygeros

Nature Biotechnology Vol, 29 n°13 (December 2011)

Realization of basic modules of a control scheme

- **One possible CRN for subtractor realization**

$$\begin{aligned} \varnothing &\xrightarrow{\Phi_A} A \xrightarrow{k_1} C \\ \varnothing &\xrightarrow{\Phi_B} B \\ A + B &\xrightarrow{k_2} W\,. \end{aligned} \qquad (1)$$

The model of CRN (1) is given by

$$\begin{aligned} \dot{a} &= \Phi_A - k_1\, a - k_2\, a\, b \\ \dot{b} &= \Phi_B - k_2\, a\, b \\ \dot{w} &= k_2\, a\, b \\ \Phi_C = \dot{c} &= k_1\, a\,, \end{aligned}$$

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.

C. Cosentino, M. Bilotta, A. Merola, and F. Amato "A Synthetic Biology Approach to the Realization of Embedded Feedback Controllers for Chemical Reaction Networks", 13th IEEE International Conference on BioInformatics and BioEngineering **(BIBE), November 10-13, Chania, Greece**

Realization of basic modules of a control scheme

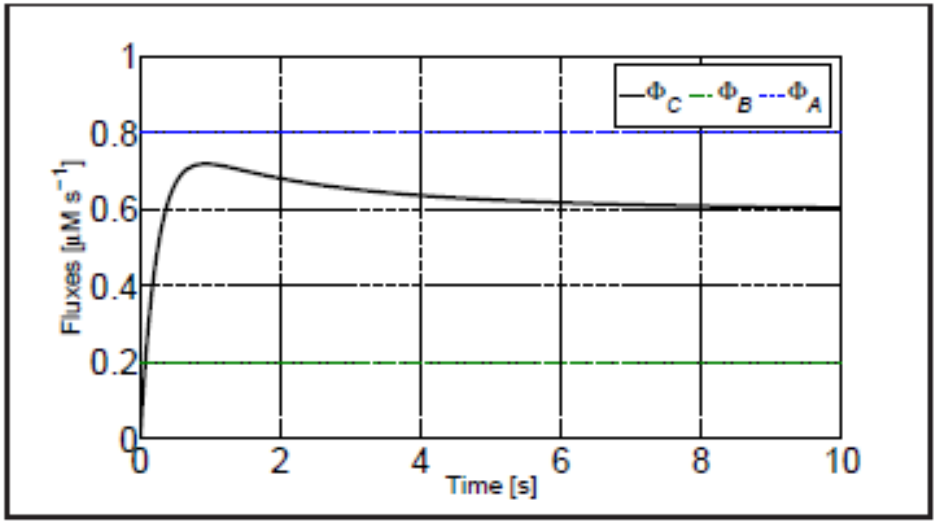


Fig. 2. Response of the isolated subtractor (CRN (1)) with the input fluxes given by $\Phi_A = 0.8\,\mu M\,s^{-1}$ and $\Phi_B = 0.2\,\mu M\,s^{-1}$, and the kinetic parameters set to $k_1 = 4\,s^{-1}$ and $k_2 = 3\,(\mu M\,s)^{-1}$ to have a settling time by $10\,s$. The output flux (solid line) Φ_C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

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Realization of basic modules of a control scheme

- **Amplifier**

$$\varnothing \xrightarrow{u_A} A \xrightarrow{k_1} A + C$$
$$B + C \xrightarrow{k_2} B$$
$$C \xrightarrow{k_3} \varnothing .$$

$$u = u_A$$
$$y = \dot{c}$$

Mathematical model:

$$\dot{a} = u_A$$
$$\dot{b} = 0$$
$$\dot{c} = k_1 a - k_2 bc - k_3 c$$

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→A+C with rate k_1 might equivalently be replaced by A + Z→ 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.

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Realization of basic modules of a control scheme

Laplace trasform:

$$s\,a(s) = u_A(s)$$

$$y(s) = s\,c(s) = k_1 a(s) - k_2 \bar{b} c(s) - k_3\, c(s$$

Transfer function: $\frac{y(s)}{u_A(s)} = \frac{k_1}{(s+k_3+k_2\bar{b})}$

Proportional gain: $K_P = \frac{k_1}{k_3+k_2\bar{b}}$

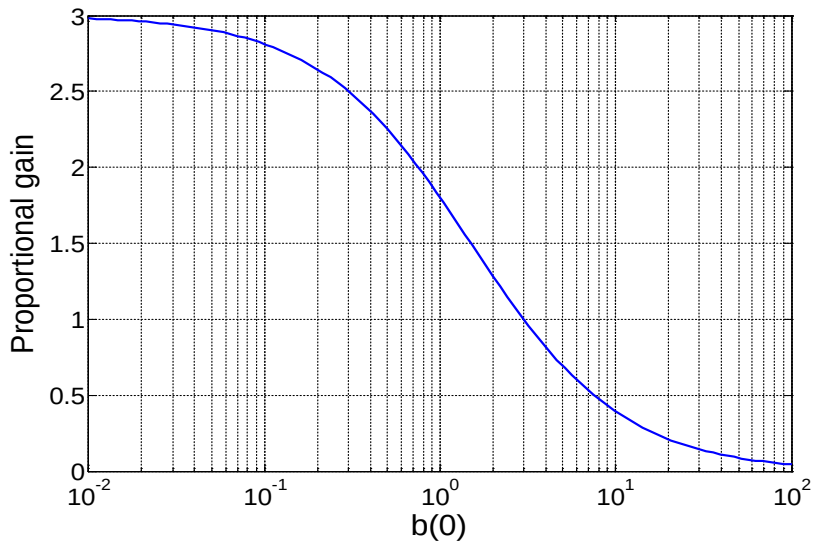

Fig. 4: Proportional gain of the amplification module.

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Case study: control of a simple enzymatic CRN

- **Process:** Michaelis-Menten irreversible enzymatic reaction:

$$u = u_S$$
$$y = \dot{p}$$

$$\xrightarrow{u_S} S + E \underset{k_r}{\overset{k_f}{\rightleftharpoons}} E:S \xrightarrow{k_{cat}} E + P$$

where $E:S$ denotes the formation of a complex C, enzyme-substrate.

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.

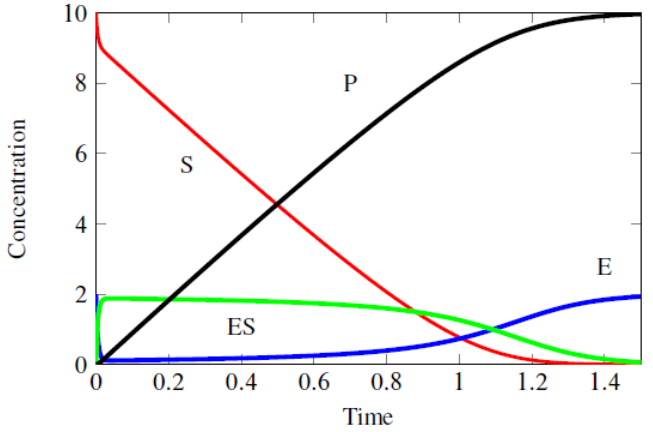
C. Cosentino, M. Bilotta, A. Merola, and F. Amato "A Synthetic Biology Approach to the Realization of Embedded Feedback Controllers for Chemical Reaction Networks", 13th IEEE International Conference on BioInformatics and BioEngineering **(BIBE), November 10-13, Chania, Greece**

Case study: control of a simple enzymatic CRN

- **Process:** Michaelis-Menten irreversible enzymatic reaction

Mathematical model:

$$\dot{s} = k_r\, c - k_f\, s\, e + u_S$$
$$\dot{c} = -(k_r + k_{cat})\, c + k_f\, s\, e$$
$$\dot{e} = (k_r + k_{cat})\, c - k_f\, s\, e$$
$$\dot{p} = k_{cat}\, c$$



The steady-state values:

$$\bar{s} = \frac{(k_r + k_{cat})\bar{u}}{k_f k_{cat} \bar{e}} \qquad \bar{c} = \frac{\bar{u}}{k_{cat}} \qquad \bar{e} = e_{tot} - \frac{\bar{u}}{k_{cat}}$$

where $e_{tot}(t) = c(t) + e(t)$.

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Proportional control of a simple enzymatic CRN

Proportional controller

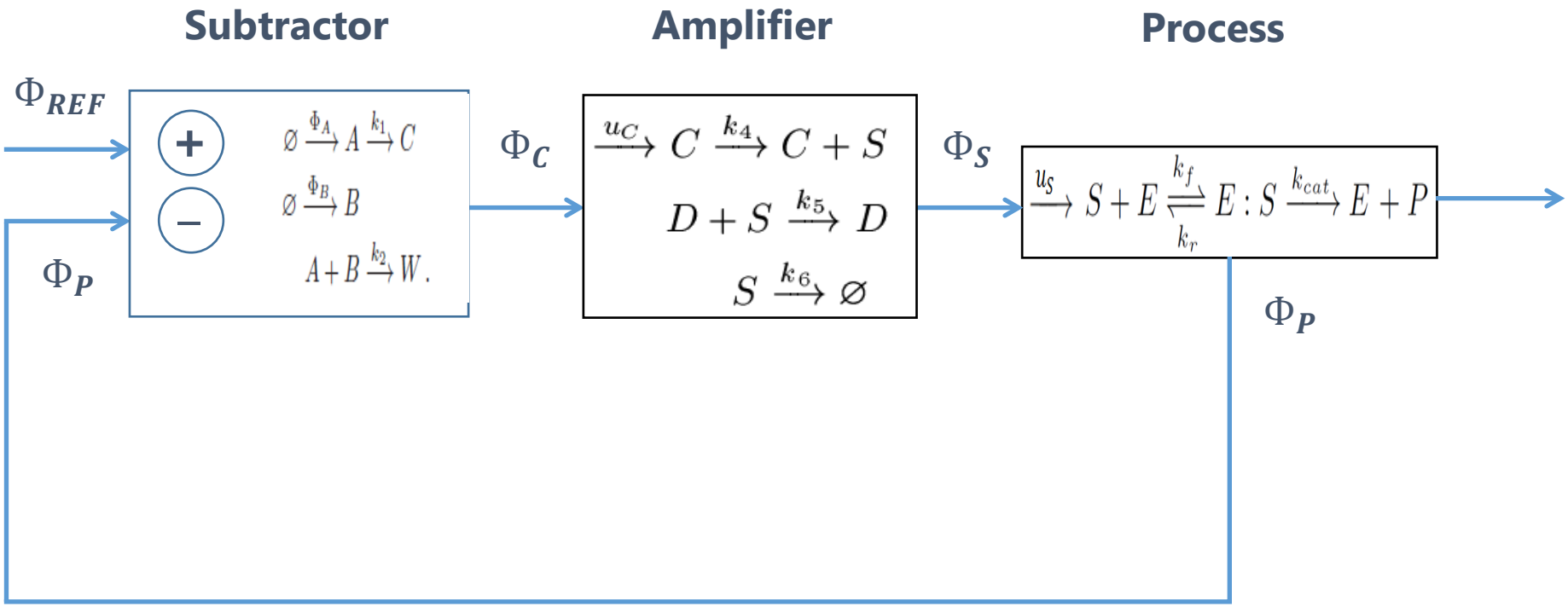

Fig. 5: Interconnection of three CRN-based modules to realize an embedded feedback controller for a CRN plant.

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Controller design

Requirements for the linearized closed-loop system:

- Settling time at 1% of the final value
  $T_s < 35\ s$
- GM > 10 dB ,
- PM > 120°

The desired output can be achieved by injecting

$$\bar{u} = 0.2\ \mu M\ s^{-1}$$

Steady–state equilibrium:

$$\bar{s} = 0.88\ , \bar{c} = \frac{1}{3}\ , \bar{e} = \frac{1}{6}$$

$$\bar{y} = \bar{u}$$

Substraction Module		
$k_1 = 300\ s^{-1}$	$k_2 = 2\ s^{-1}$	$k_3 = 1\ \mu M^{-1} s^{-1}$

Amplification Module		
$k_4 = 9\ s^{-1}$	$k_5 = 2\ \mu M^{-1} s^{-1}$	$k_6 = 3\ s^{-1}$

Plant			
$k_f = 15\ \mu M^{-1} s^{-1}$	$k_r = 6\ s^{-1}$	$k_{cat} = 0.6\ s^{-1}$	$e_{tot} = 0.5\ \mu M$

A settling time $T_s = 32.2\ s$, robustness margins $GM = \infty$ and $PM = \infty$ can be guarenteed for $K_P = 0.8$

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Result of a proportional control

Control goal : $y_r = 0.23\ \mu M\ s^{-1}$

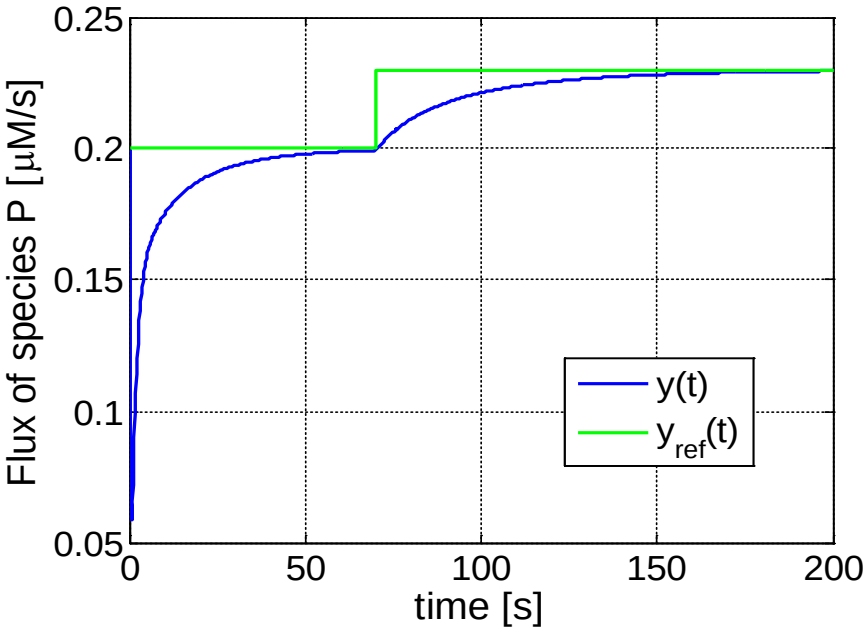

Fig. 6: System response to a step input.

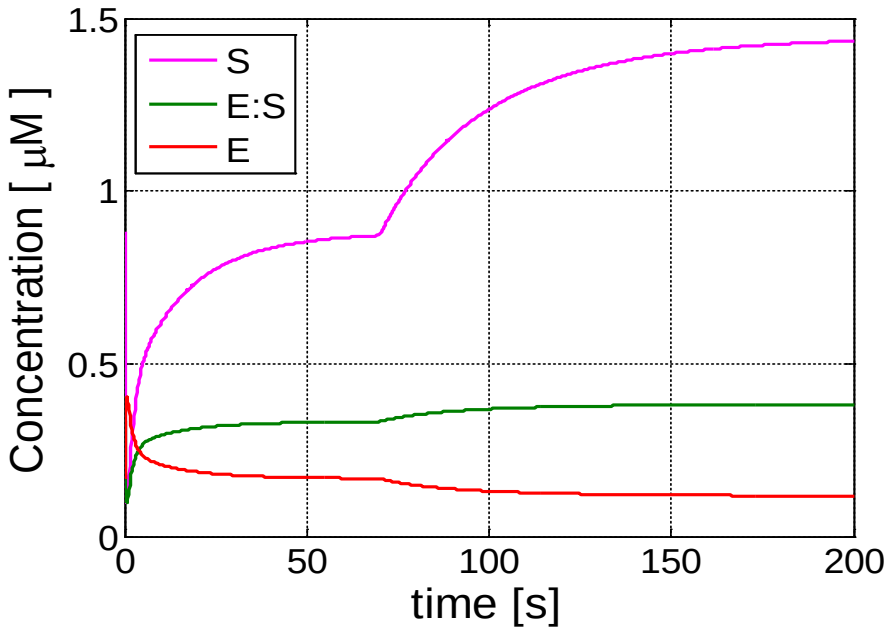

Fig. 7: Evolution of the species involved in the controlled CRN.

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Important topics do not discussed here

- PI control.
- *Retroactivity* issues.
- *In vitro* experiments

C. Cosentino, M. Bilotta, F. Montefusco, R. Sawlekar, F. Amato and D. Bates *A Modular Approach to the Design of Embedded Controllers for Chemical Reaction Networks*. Synthetic Biology: Engineering, Evolution & Design **(SEED) Conference, July 14th-17th 2014, Manhattan, CA**

M. Bilotta, C. Cosentino, D. G. Bates, F.Amato *Retroactivity Analysis of a Chemical Reaction Network Module for the Subtraction of Molecular Fluxes.* **37th Conference of IEEE Engineering in Medicine and Biology Society, August 25th-29th 2015, Milan, IT**

Conclusions

- A novel synthetic biology approach has been proposed to realize embedded feedback controllers for CRNs.
- Basic CRN modules to design output feedback control schemes: amplifier, subtraction (and integration) modules.
- 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 embedded CRN controllers.