

PID Control of Biochemical Reaction Networks

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Abstract—Principles of feedback control have been shown to naturally arise in biological systems and successfully applied to build synthetic circuits. In this work we consider Biochemical Reaction Networks (CRNs) as a paradigm for modelling biochemical systems and provide the first implementation of a derivative component in CRNs. That is, given an input signal represented by the concentration level of some species, we build a CRN that produces as output the concentration of two species whose difference is the derivative of the input signal. By relying on this component, we present a CRN implementation of a feedback control loop with Proportional-Integral-Derivative (PID) controller and apply the resulting control architecture to regulate the protein expression in a *microRNA* regulated gene expression model.

I. INTRODUCTION

Biochemical Reaction Networks (CRNs) are a widely used formalism to describe biochemical systems [1]. More recently, they have also been employed as a formal programming language for synthetic circuits made of DNA [2], [3]. Due to the numerous potential applications, ranging from smart therapeutics to biosensors, the construction of CRNs that exhibit prescribed dynamics is a major goal of synthetic biology. However, achieving a desired behaviour by designing a CRN is difficult due to the complexity of such systems and limited knowledge of their dynamics [4], [5].

Negative feedback and Proportional-Integral-Derivative (PID) control are widely used in engineering to control the dynamics of a system due to their ability to achieve accurate set-point tracking and robustness to disturbances, even with only partial knowledge of the system. Because of these properties, such mechanisms have also been applied with success in the construction of synthetic bio-molecular systems [6], [7]. Moreover, molecular implementation of control systems has been shown to naturally occur in living organisms [8], [9], [10]. For example, integral control occurs in *E.coli* chemotaxis [11], [12], while *CheY* proteins regulate the bacteria's tumbling frequency by implementing a derivative control [13]. As a consequence, in view of the potential applications, CRN designs that implement control mechanisms are sought for [12], [6]. CRNs implementing proportional and integral control have been proposed [14], [12]. However, a CRN implementation of a full PID control is still missing due to the lack of a CRN implementing the derivative component. In [15] a biologically inspired

implementation of a system that computes the derivative of an input is provided, however it is presented as non-mass action Hill functions. We motivate the use of CRNs with mass action kinetics given that any CRN with mass action kinetics has a translation scheme to an equivalent DNA Strand Displacement Device [16], [17].

In this work we first present a CRN implementation of a derivative component. That is, we provide a CRN such that, given an input signal represented by the concentration level of some species, the output is the concentration of two species whose difference gives the derivative of the input signal. We use this as a building block for a PID controller, and show how negative feedback with PID controller can be implemented in CRNs. We show the effectiveness of this architecture on a *microRNA* regulated gene expression example [18], [19], where we control the time evolution of a protein by acting on the expression of mRNA and *microRNA*.

In summary, we make the following contributions:

- We present a CRN that computes the derivative of an input signal and prove its asymptotic correctness.
- We extend the correctness results of CRN encodings [14] of proportional and integral signals to the case of nonlinear dynamics.
- We provide for an arbitrary CRN plant a CRN encoding of the PID feedback controller.
- We show the effectiveness of our control architecture on a *microRNA* regulated gene expression model.

II. BIOCHEMICAL REACTION NETWORKS

In this section we provide some background about the deterministic mass-action semantics of a CRN based on the reaction-rate equations. Then we review the notion of dual rail encoding for a species. Finally we fix a graphical representation of CRNs that will be used throughout the paper.

A. Deterministic Mass-action semantics

A CRN $\mathcal{C} = (\mathcal{S}, \mathcal{R})$ is a pair of finite sets, where \mathcal{S} is an ordered set of species, $|\mathcal{S}|$ denotes its size, and \mathcal{R} is an ordered set of reactions. Species in \mathcal{S} interact according to the reactions in \mathcal{R} . A reaction $\tau \in \mathcal{R}$ is a triple $\tau = (r_\tau, p_\tau, k_\tau)$, where $r_\tau \in \mathbb{N}^{|\mathcal{S}|}$ is the *reactant complex*, $p_\tau \in \mathbb{N}^{|\mathcal{S}|}$ is the *product complex* and $k_\tau \in \mathbb{R}_{>0}$ is the coefficient associated with the rate of the reaction. Complexes r_τ and p_τ represent the stoichiometry of reactants and products. We denote the i -th component of complex r_τ by $r_{\tau,i}$; the zero complex is denoted by \emptyset . Given a CRN with species set $\mathcal{S} = \{A, B, C\}$, a reaction $([1, 1, 0], [0, 0, 2], k_1)$ will be denoted by $A + B \xrightarrow{k_1} 2C$.

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We consider the deterministic interpretation of a CRN based on the well-known reaction-rate equations with mass-action kinetics. Given a CRN $\mathcal{C} = (\mathcal{S}, \mathcal{R})$ and an initial condition $x_0 \in \mathbb{R}_{\geq 0}^{|\mathcal{S}|}$ representing the initial concentration of each species, the time course of the concentrations can be described as the solution of an initial value problem with the following system of ODEs

$$\partial_t x(t) = \sum_{(r_\tau, p_\tau, k_\tau) \in \mathcal{R}} (p_\tau - r_\tau) k_\tau \prod_{i=1}^{|\mathcal{S}|} x_i(t)^{r_{\tau,i}}, \quad (1)$$

and initial condition $x(0) = x_0$. For a species $A \in \mathcal{S}$ we denote by $x_A(t)$, or x_A when the time dependence is clear from the context, the concentration of A at time t .

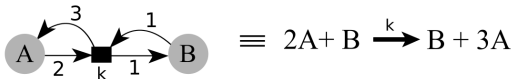
In this paper we synthesise PID controllers with mass-action kinetics for CRNs. We will also assume that the plant is represented by a mass-action CRN, although our results carry over to plants given in terms of smooth control systems.

B. Dual Rail Encoding

The plant is a CRN, hence its output is given by non-negative solutions. However, the PID controller will involve quantities that are negative such as the error, i.e., difference between the set-point and the output, as well as its derivative. In order to handle this we will use the so-called dual rail encoding [14], by which a signal is decomposed into a “positive” and “negative” species component whilst preserving a law of mass action kinetics such that each individual species concentrations cannot be negative. Specifically, for a signal A we denote the two distinct component species by A^+ and A^- , representing A as the difference $A^+ - A^-$. An annihilation reaction of the form $A^+ + A^- \rightarrow \emptyset$ keeps those species in check while not affecting their difference. Owing to the fact that the plant is described by a mass-action CRN, and therefore concentrations of species are strictly positive, we wish to point out that its non-negative output is captured by a single rail encoding.

C. Graphical Representation of CRNs

A CRN can be represented as a labelled directed bipartite graph according to the usual Petri net representation with species and reaction nodes [20]. A reaction node is labelled with a rate coefficient. There is an edge from a species node to a reaction node if the species is in the reactant complex, with label equal to its multiplicity; similarly, an edge from a reaction node to a species node indicates the presence of a species in the product complex. For example, we have the following representation for the reaction $2A + B \xrightarrow{k} B + 3A$:



Throughout the rest of the paper, for ease of presentation we do not draw labels on edges if the related complex multiplicity is 1. We also remove the black box representing reaction nodes to reduce clutter. Finally, we introduce a short-hand notation for recurring reaction patterns as shown in Figure 1, where each arc is either a pointed arrow (\rightarrow) or a rounded arrow (\curvearrowright),

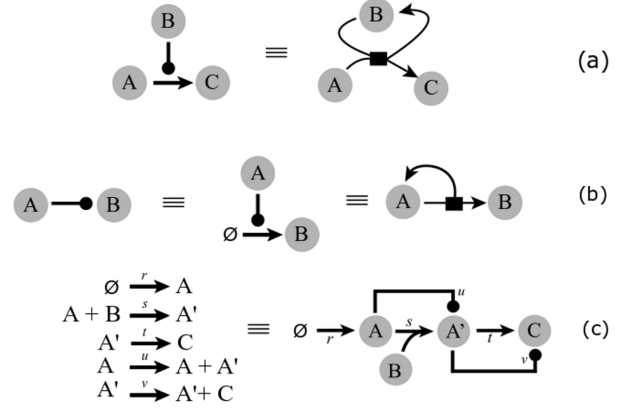


Fig. 1: Short-hand CRN graphical notation. (a) A catalytic bi-molecular reaction $A + B \rightarrow B + C$ as an equivalent Petri net; (b) a catalytic uni-molecular reaction $A \rightarrow A + B$; (c) a sample CRN depicted using the short-hand notation.

with the source represented by the flat edge and the target represented by the arrow head. A pointed arrow represents a non-catalytic reaction and a rounded arrow represents a catalytic reaction.

III. CRN IMPLEMENTATION OF THE PID CONTROLLER

We introduce the CRN implementation of the proportional, integral, and derivative components of a PID controller. We describe them as blocks where the input species are E^\pm (which will indicate the dual-rail error signal between the species representing the set-point and the plant output). The output of the PID controller is denoted by U^\pm . The proportional and integral components have been already introduced for linear control systems [14]. Here we prove their correctness in the presence of non-linearity. In addition we detail the CRN implementation of the derivative block, which is a novel contribution to the best of our knowledge.

As shown in Figure 2, the signals synthesised by the PID controller act on the CRN plant, whose output is measured, and sent back as input of the PID controller after comparison with the reference signal. The output of the plant is always given by a species Y . The objective of the control is to have Y follow the reference signal.

To this end, we let $(\mathcal{S}_\Sigma, \mathcal{R}_\Sigma)$ denote the mass-action CRN representing the plant and construct a CRN encoding of a PID feedback law as indicated in Figure 2. For the benefit of presentation, we focus on one-dimensional controls. However, as will be discussed later, the discussion naturally generalises to the multidimensional case. The CRN encoding is given by

- subtraction block $(\mathcal{S}^S, \mathcal{R}^S)$
- addition block $(\mathcal{S}^A, \mathcal{R}^A)$
- proportional block $(\mathcal{S}^P, \mathcal{R}^P)$
- integral block $(\mathcal{S}^I, \mathcal{R}^I)$
- derivative block $(\mathcal{S}^D, \mathcal{R}^D)$

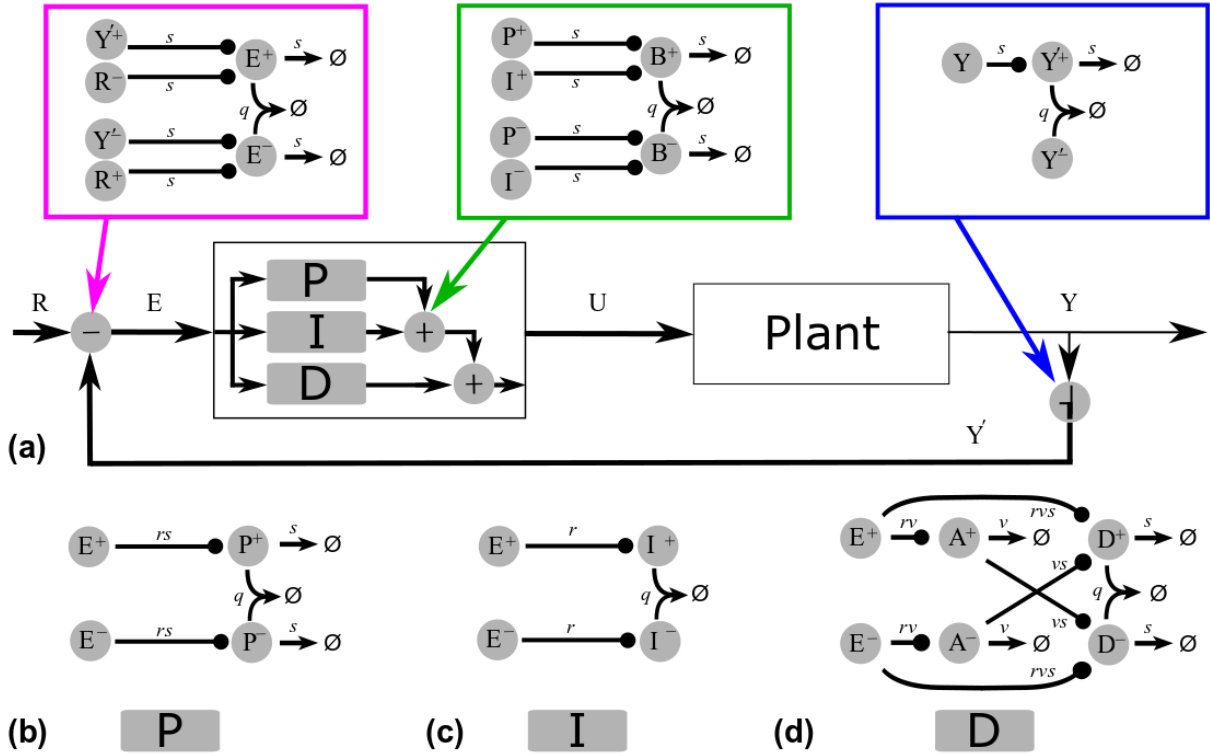


Fig. 2: We present our feedback loop (a) which takes a smooth reference signal R (in dual rail in its most general case) and, along with the feedback Y' , produces an error E (computed $Y' - R$). Signal E is obtained by the subtraction block (in magenta). Thick arrows imply dual rail whereas the thin arrow Y implies single rail. E is fed to the controller, the chemical composition of which is described in (b),(c),(d). The proportional and integral blocks (b),(c) are taken from [14]. The proportional block adjusts the input x_A as rx_A for some multiplier $r \geq 0$. The integral block takes an input x_A and produces $r \int_0^t x_A(\tau) d\tau$ for some multiplier $r \geq 0$. Instead, the novel derivative block (d) takes an input x_A and produces the output $r \partial_t x_A$, where $r \geq 0$ is a multiplier. The foregoing blocks are summed by the addition block (in green), yielding a control signal U which steers the plant by the CRN encoding presented in Section III. As a result, the plant produces a signal Y which is converted to a dual rail signal Y' by the dual rail converter block (in blue). The presence of a multiplier in each block allows one to adjust the weights of each block. In particular, parameter values r, s, v, q are block dependent in general.

- dual rail converter block $(\mathcal{S}^C, \mathcal{R}^C)$.¹

With this, the overall CRN is given by

$$(\mathcal{S}_\Sigma, \mathcal{R}_\Sigma) \cup (\mathcal{S}_F, \mathcal{R}_F), \quad (2)$$

where the feedback law CRN is defined by

$$(\mathcal{S}_F, \mathcal{R}_F) = \bigcup_{X \in \mathcal{X}} (\mathcal{S}^X, \mathcal{R}^X), \quad \mathcal{X} = \{S, A, P, I, D, C\}.$$

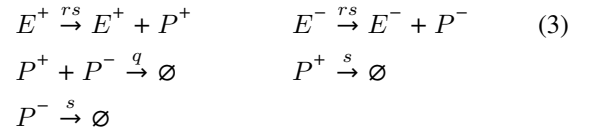
In what follows next, we address the correctness of each block type. Each block is responsible for providing the annihilation reactions only for the new species that it introduces.

A. Proportional, Addition, Subtraction and Dual Rail Converter Blocks

We first present the proportional block which computes the output signal that is proportional to the input signal.

¹The presence of P , I and D blocks is without loss of generality because a block can be removed by setting its multiplier r to zero.

Definition 1 (Proportional block [14]). *For input species E^+, E^- , output species P^+, P^- , parameters $s, q \in \mathbb{R}_{>0}$, and the multiplier $r \in \mathbb{R}_{\geq 0}$, the proportional block is a CRN composed by the following reactions*



We show the asymptotic correctness of our blocks by applying Tikhonov's theorem [21, Section 8.2] to the ODE system underlying the CRN (2). This amounts to conducting a rigorous quasi steady-state approximation [22] where ODE variables are partitioned into *fast* and *slow* ones. With the intuition being that fast variables attain their equilibria almost instantaneously compared to slow variables, the approximation suggests to drop the ODEs of fast variables and to replace the fast variables by their equilibrium values (which, in general, will depend on the values of slow variables).

When applying Tikhonov's theorem, it is convenient to

call the ODEs of fast and slow variables as the fast and slow ODE system, respectively. In our proofs, the main objective is to identify the slow and fast ODE systems and to show that the requirements of Tikhonov's theorem are satisfied.

Theorem 1. *On any bounded time interval, the solution of the ODE system induced by the CRN (2) converges, as $s \rightarrow \infty$ in (3), to a solution satisfying $x_{P^+} = rx_{E^+}$ and $x_{P^-} = rx_{E^-}$.*

Proof (Sketch). Consider

$$\begin{aligned}\partial_t x_{P^+} &= r s x_{E^+} - s x_{P^+} - q x_{P^+} x_{P^-} \\ \partial_t x_{P^-} &= r s x_{E^-} - s x_{P^-} - q x_{P^+} x_{P^-}\end{aligned}\quad (4)$$

We interpret x_{P^+} and x_{P^-} as fast variables in the sense of Tikhonov's theorem and all other variables in CRN (2) as slow ones. Our claim readily follows if we can establish the requirements of Tikhonov's theorem, see [21, Section 8.2]. To see that those are indeed satisfied, we note that the fast ODE system (4) admits, for any fixed vector of slow variables, exactly one possible equilibrium point. Moreover, it is an asymptotically stable equilibrium of the fast ODE system. We finish the proof by noting that smooth exogenous reference signals can be captured because Tikhonov's theorem applies to non-autonomous smooth ODE systems. \square

Several remarks are in order.

Remark 1. *By saying that $s \rightarrow \infty$ in (3), we mean that only the s variable in the proportional block approaches infinity, while the s variables in all other blocks are held fixed. This abuse of notation can be fixed, at the price of heavier notation, by the use of pairwise different variables in each block. That is, instead of using r , s and q in every block, one would use r^X , s^X and q^X , where $X \in \{S, A, P, I, D, C\}$ refers to the respective block as in the definition of (S_F, R_F) .*

Remark 2. *For the benefit of presentation, our discussion focuses on the case of one-dimensional controls. We wish to stress however that it easily generalises to n -dimensional controls. Indeed, all one has to do is to index the variables by the subscript $1 \leq i \leq n$. For instance, the proportional block becomes $(S^P, R^P) = \bigcup_{i=1}^n (S_i^P, R_i^P)$, where (S_i^P, R_i^P) arises from (3) by replacing r with r_i and by adding the subscript i to each species (e.g., E^+ becomes E_i^+). Similar statements apply to all other blocks.*

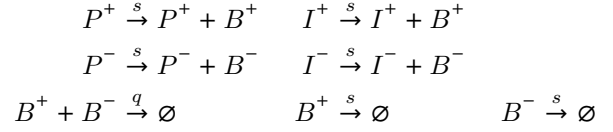
Remark 3. *Theorem 1 extends the result of [14] to arbitrary (nonlinear) CRN plants. The same holds true for the other blocks of this section.*

Remark 4. *The proof of Theorem 1 reveals that reaction $P^+ + P^- \xrightarrow{q} \emptyset$ is not strictly needed to ensure correctness. However, this reaction precludes P^+ and P^- from attaining excessively large values (recall that s is large), thus reducing the impact of numerical errors.*

The correctness of the subtraction and converter blocks introduced next and depicted in Figure 2 is shown similarly to Theorem 1.

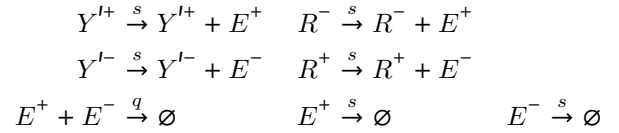
More specifically, the addition block is given by.

Definition 2 (Addition block [14]). *For input species P^+, I^+ and P^-, I^- , output species B^+, B^- , and parameters $s, q \in \mathbb{R}_{>0}$ the addition block is a CRN composed by the following reactions*



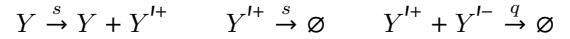
The subtraction block, instead, is defined as follows.

Definition 3 (Subtraction block [14]). *For input species Y^{I^+}, R^+ and Y^{I^-}, R^- , output species E^+, E^- , and parameters $s, q \in \mathbb{R}_{>0}$ the subtraction block is a CRN composed by the following reactions*



At last, the converter block is described by the following.

Definition 4 (Dual rail converter block). *For input species Y , output species Y^{I^+}, Y^{I^-} , and parameters $s, q \in \mathbb{R}_{>0}$ the single to dual rail converter block is a CRN composed by the following reactions*

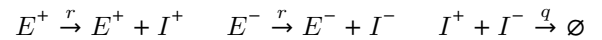


Note that being Y a positive signal, the dual rail converter block simply copies Y in Y^{I^+} . The annihilation reaction $Y^{I^+} + Y^{I^-} \xrightarrow{q} \emptyset$ is needed just in case initial concentration of Y^{I^-} is non-zero.

B. Integral Block

The integral component computes a multiple of the integral of the input signal. This action is widely used in control systems due to its ability to collect past information about the error to be corrected. A CRN implementation of the integral component has been proposed in [14] and is reported in Definition 5.

Definition 5 (Integral block [14]). *For input species E^+, E^- , output species I^+, I^- , some constant $q \in \mathbb{R}_{>0}$ and multiplier $r \in \mathbb{R}_{\geq 0}$, the integral block is given by the following CRN*



Proposition 1. *The integral block introduced in Definition 5 is correct. More formally, it holds that*

$$x_{I^+}(t) - x_{I^-}(t) = r \int_0^t x_{E^+}(\tau) d\tau - r \int_0^t x_{E^-}(\tau) d\tau$$

Proof. Straightforward via differentiation (the values at $t = 0$ are chosen appropriately). \square

Proposition 1 is a (straightforward) extension of the corresponding result in [14] to arbitrary (nonlinear) CRN plants.

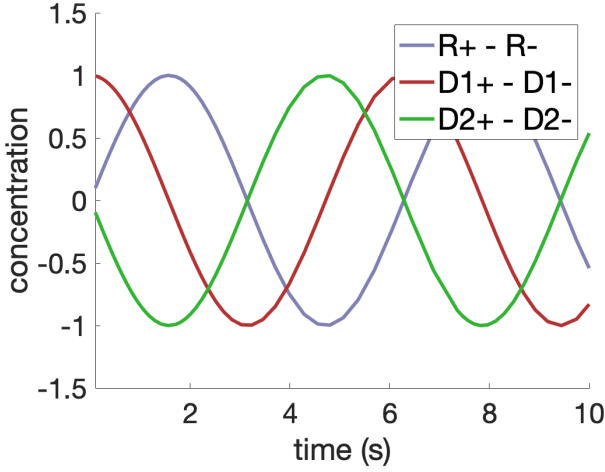


Fig. 3: The output of two successive derivative blocks against an input of a sine wave. $D1^+$, $D1^-$ refers to the output of the first derivative block whilst $D2^+$, $D2^-$ refers to the output of the second. It can be observed that the output of the second derivative block is a cosine wave.

C. Derivative Block

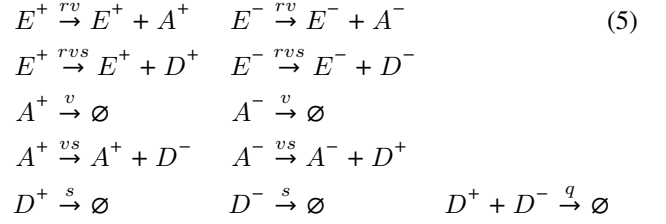
The derivative block computes a multiple of the derivative of the input signal. This component is used in control systems to predict the future error given its current trend, and thus to help dampen oscillations introduced by P and I components.

Building a derivative module by chemical reactions is challenging because on-the-fly differentiation can only be done by comparing a signal at two time points, inherently requiring an approximation dependent on the time difference. This is resolved by the circuit in Figure 2(d), which handles dual rail input and output. Intuitively, the inputs E^+ and E^- are sampled at two time points, E^+ , A^+ and E^- , A^- , respectively, and a multiple of their difference is provided via D^+ , D^- . In Figure 2(d), the two reactions $E^+ \xrightarrow{rv} E^+ + A^+$ and $A^+ \xrightarrow{v} \emptyset$ cause x_{A^+} to track rx_{E^+} with a (slight) delay dependent on v . Intuitively, this yields $rx_{E^+} - x_{A^+} \approx \partial_t rx_{E^+}$. The symmetric two reactions similarly cause x_{A^-} to track rx_{E^-} , which ensures that $rx_{E^-} - x_{A^-} \approx \partial_t rx_{E^-}$. The three reactions $E^+ \xrightarrow{rvs} E^+ + D^+$, $A^- \xrightarrow{vs} A^- + D^+$, and $D^+ \xrightarrow{s} \emptyset$ cause x_{D^+} to track $rvx_{E^+} + vx_{A^-}$ with delay dependent on s . The symmetric three reactions similarly cause x_{D^-} to track $rvx_{E^-} + vx_{A^+}$. Thus $x_{D^+} - x_{D^-}$ tracks $v(rx_{E^+} + x_{A^-}) - v(rx_{E^-} + x_{A^+}) = v(rx_{E^+} - x_{A^+}) - v(rx_{E^-} - x_{A^-})$ with delay dependent on s . This and the above discussion allow us then to conclude that $x_{D^+} - x_{D^-} \approx r\partial_t(x_{E^+} - x_{E^-})$. We can observe an example behaviour of this derivative block in Figure 3 which computes the cosine function with a sine function as input.

The next theorem formalises the above considerations using Tikhonov's theorem.

Definition 6. For input species E^+, E^- , auxiliary species A^+, A^- , output species D^+, D^- , parameters $q, s, v \in \mathbb{R}_{>0}$ and the multiplier $r \in \mathbb{R}_{\geq 0}$, the derivative block is a CRN

composed by the following reactions



The following theorem shows that the above CRN is such that, under certain scaling of the rates, $(x_{D^+} - x_{D^-})$ produces a correct approximation of the derivative of $r(x_{E^+} - x_{E^-})$.

Theorem 2. The derivative block is asymptotically correct. In particular, the following holds.

- 1) The ODE solution of (2) converges, on any bounded time interval, to an ODE system satisfying $x_{D^+} - x_{D^-} = \partial_t x_{A^+} - \partial_t x_{A^-}$ when $s \rightarrow \infty$ in (5).
- 2) The ODE solution of (2) converges, on any bounded time interval, to an ODE system satisfying $x_{A^+} = rx_{E^+}$ and $x_{A^-} = rx_{E^-}$ when $v \rightarrow \infty$ in (5).

Proof (Sketch). To see 1), we first note that Definition 6 yields

$$\begin{aligned}
 \partial_t x_{D^+} &= svrx_{E^+} + vsx_{A^-} - sx_{D^+} - qx_{D^+}x_{D^-} \\
 \partial_t x_{D^-} &= svrx_{E^-} + vsx_{A^+} - sx_{D^-} - qx_{D^+}x_{D^-} \\
 \partial_t x_{A^+} &= vrx_{E^+} - vx_{A^+} \\
 \partial_t x_{A^-} &= vrx_{E^-} - vx_{A^-}
 \end{aligned}$$

Since this implies

$$\begin{aligned}
 \partial_t(x_{D^+} - x_{D^-}) &= s(vrx_{E^+} - vx_{A^+}) \\
 &\quad - s(vrx_{E^-} - vx_{A^-}) - s(x_{D^+} - x_{D^-}),
 \end{aligned}$$

this motivates us to add to the ODE system of (2) the additional ODE

$$\partial_t z = s \underbrace{(vrx_{E^+} - vx_{A^+})}_{=\partial_t x_{A^+}} - s \underbrace{(vrx_{E^-} - vx_{A^-})}_{=\partial_t x_{A^-}} - sz$$

and to set $z(0) = (x_{D^+} - x_{D^-})(0)$, thus ensuring that $z \equiv x_{D^+} - x_{D^-}$. To see that the requirements of Tikhonov's theorem are satisfied, we note that the fast ODE system (with the fast variables being z , D^+ and D^-) admits, for any fixed vector of slow variables, exactly one equilibrium point. Additionally, it is an asymptotically stable equilibrium of the fast ODE system. To see 2), instead, we apply Tikhonov's theorem in the case where x_{A^+} and x_{A^-} are treated as fast variables (while all other variables are considered to be slow) and let $v \rightarrow \infty$. \square

IV. PID CONTROL OF GENE EXPRESSION

In this section we apply the PID feedback control architecture developed in this paper to a gene expression model. In particular, we consider a *microRNA* regulated gene expression model from [19], for which synthetic implementations have already been proposed in [23]. The model is composed

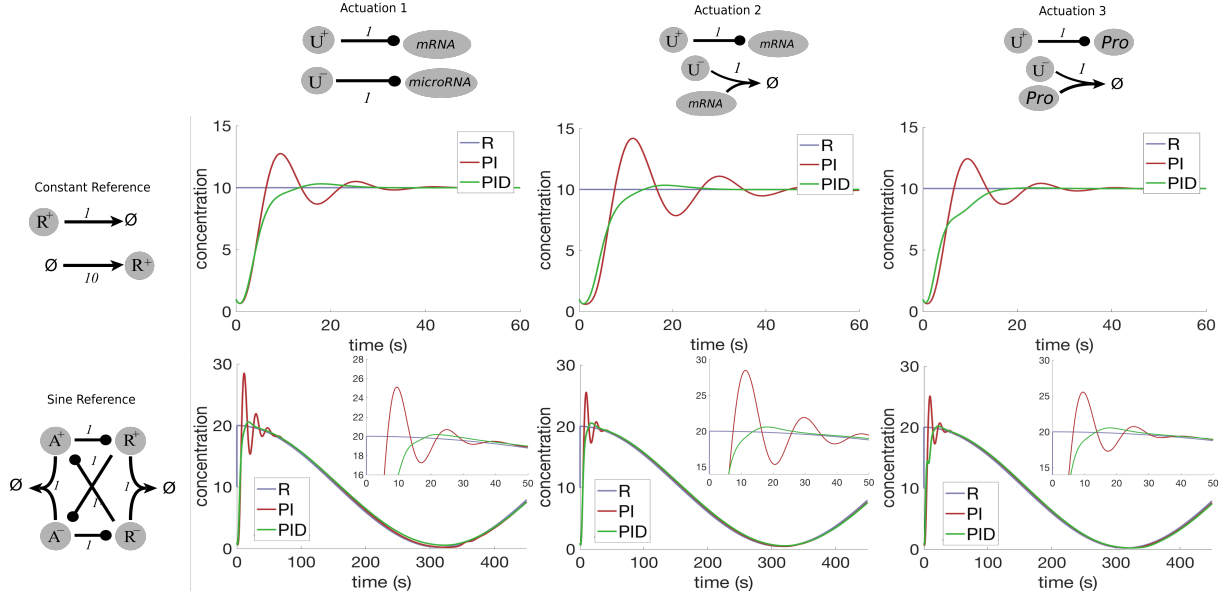
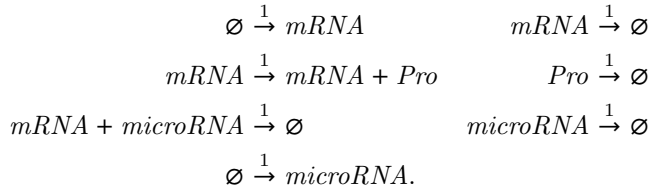


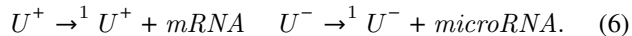
Fig. 4: We consider the gene expression model given in Section IV and compare the time evolution of the species *Pro* with different reference signals for PID and PI feedback control with the actuation models described in Equations (6), (7), and (8) shown in the left, middle, and right column of the plots, respectively. We consider both a constant and a sine wave reference signal (shown in top and bottom rows of the plots, respectively). It is possible to observe that while *Pro* already tracks correctly the reference signals for PI control, in the case of a PID controller, the output has reduced oscillations around the reference signal. This is emphasised in the insets seen on the sine reference row where we examine the first 50 seconds of the time evolution.

by the following reactions, where for simplicity we fixed unitary kinetic parameters



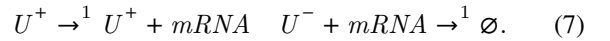
That is, we have that *mRNA* catalyses the production of the protein *Pro* and is down-regulated by an annihilation reaction with the *microRNA*.

The objective of our control is to have the protein *Pro* to follow a reference signal. Given U^+ and U^- , the control signals synthesised by the controller, we assume that these can act on the plant by regulating the expression rate of *mRNA* and *microRNA*, respectively. This assumption is justified by the fact that these mechanisms can be implemented synthetically [23]. We consider the following reactions to model such actuation:

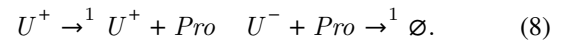


In this model, a high concentration of U^+ will increase the production rate of *mRNA* and so of *Pro*, whereas a high concentration of U^- will decrease the amount of *mRNA* by producing *microRNA* with a higher rate.

In the actuation model considered above we have that the control signals act on two different species. This is not a requirement of our architecture. Another possible actuation is that U^- annihilates *mRNA* directly. This can be modelled with the following reactions



Finally, another possibility is that U^+ and U^- acts directly on the target species *Pro*. In this case, the actuation is



In Figure 4 we consider the different actuation mechanisms described above and compare the performance of PI and PID controllers for two different reference signals: a constant signal and an oscillatory signal. For all the plots in the figure we considered the same parameters for PI and PID controllers (reported in the Appendix). It is easy to observe that, whereas a negative feedback with PI controller can already track both signals correctly, in the case of a PID controller the time evolution of the concentration of *Pro* has reduced oscillations around the reference signals. In all cases the time for the convergence of the plant to the reference signal has decreased due to the action of the derivative block. In fact, while it is well known that the derivative component in a PID does not necessarily reach zero error at steady state, it can help to

reduce the transient error between the output and the reference signals and to dampen oscillations around the set points.

V. CONCLUSION

In this work we considered feedback control with PID controllers and proposed a CRN implementation for this control architecture. This relies on a novel CRN, which computes the derivative of an input molecular signal. We applied our framework to control the protein expression in a *microRNA* regulated gene expression model and showed improved performance compared to a PI feedback control. An interesting aspect, which has not been considered in this paper, is to study the effect that the proposed control system has on noise [24]. This is left as future work.

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APPENDIX

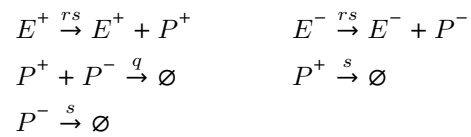
A. CRN for PID control of gene expression

We present our full Chemical Reaction Network PID feedback loop with gene expression plant and two reference signals introduced in Section IV. We include the three actuation mechanisms given with the plant. For each block we also give the initial conditions used to produce the simulations seen in Figure 3.

PID Controller

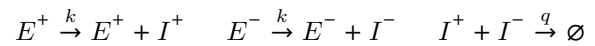
First we report the reactions and parameters of the CRN PID controller of which the proof of correctness and details of operation are outlined in Section 3. For all figures we used the same parameters. Please note that rates denoted with multiple letters are the products of those individual letters, i.e. a rate rs is the product of two rates $r \times s$.

Proportional Block:



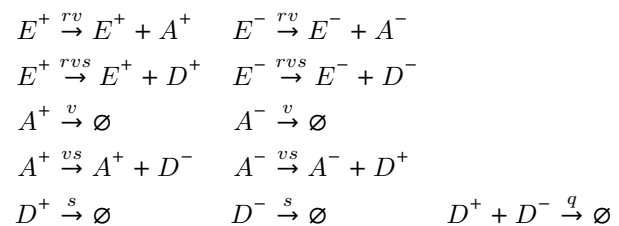
Initial Conditions: (rates) $s, rs, q = 1$ (species) $P^+, P^- = 0$

Integral Block:



Initial Conditions: (rates) $k, q = 1$, (species) $I^+, I^- = 0$

Derivative Block:

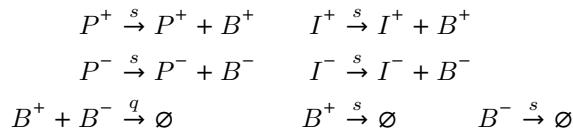


Initial Conditions: (rates) $v, s, vs = 1, rv, q, rvs = 10$, (species) $A^+, A^-, D^+, D^- = 0$

B. Summation and Subtraction Blocks

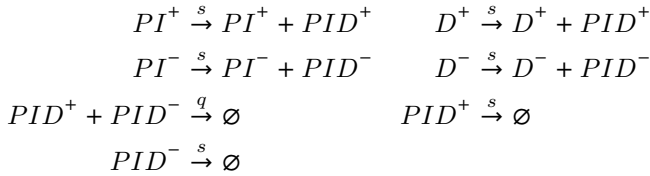
We provide the CRNs for the two summation blocks which are highlighted in the green block in Figure 2 and noted upon in section 3. The first adds the output of the P and I blocks together. The second the PI and D blocks together which produces the output species of the controller U (given as PID in the CRNs below). The rates for the summation blocks were tuned using a trial and error approach where the rates were adjusted to reduce error and reduce time to a steady-state. It is important to note that no set of parameters are best in all situations. The rates are within bounds for implementation as a DNA Strand Displacement device [17].

Addition Block $P + I$:



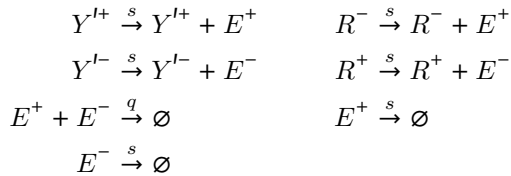
Initial Conditions for $P + I$ summation block: (rates) $s = 0.8, q = 0.3$, (species) $B^+, B^- = 0$

Addition Block $PI + D$:



Initial Paramterisation for $PI + D$ summation block: (rates) $s = 1.1, q = 0.1$ (species) $PID^+, PID^- = 0$.

Subtraction Block: The subtraction block is used to compute the error of the output of the plant Y with the reference signal. It is detailed further in Section 3:



Initial Conditions for difference block summation block: (rates) $s, q = 1$

C. Reference Signals

Next we introduce the initial conditions and reactions for both the constant and sine wave reference signals outlined further in Section 4. These act as a reference which we are trying to control our plant to track.

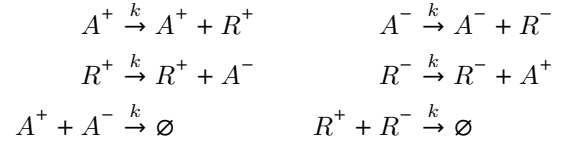
Constant:

The constant signal can be given simply by stating a non-decaying species with a molecular count equal to the constant signal however it can also be given by the following CRN which is stated in Figure 3.



Initial Conditions: (rates) $k = 10, r = 1$ (species) $R^+ = 0, R^- = 0$

Sine wave: The sine wave has a slow reaction rate to allow for the PID controller to properly track the signal.



Initial Conditions: (rates) $k = 0.01$ (species) $A^+ = 10, A^- = 0, R^+ = 0, R^- = 0$

Plant and Actuators

We introduce the gene expression plant used within our model. We also include the three actuations methods from the controller U^+, U^- seen in Figure 3 and discussed in section 4 used to interface with the plant.

Actuator 1:



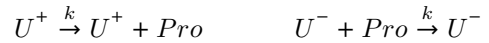
Initial Conditions: (rates) $k = 1$ (species) $mRNA = 0, microRNA = 0, U^+ = 0.5, U^- = 0.5$ where the initial values of U^+, U^- are arbitrarily set.

Actuator 2:



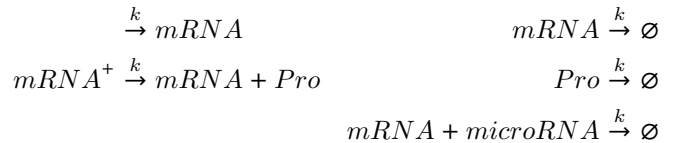
Initial Conditions: (rates) $k = 1$ (species) $mRNA = 0, U^+ = 0.5, U^- = 0.5$

Actuator 3:



Initial Conditions: (rates) $k = 1$ (species) $Pro = 1, U^+ = 0.5, U^- = 0.5$

Plant:



Initial Conditions: (rates) $k = 1$, (species) $mRNA, microRNA = 0, Pro = 1$