The Internal Model Principle for Biomolecular Control Theory

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ABSTRACT The well-known Internal Model Principle (IMP) is a cornerstone of modern control theory. It stipulates the necessary conditions for asymptotic robustness of disturbance-prone dynamical systems by asserting that such a system must embed a subsystem in a feedback loop, and this subsystem must be able to reduplicate the dynamic disturbance using only the regulated variable as the input. The insights provided by IMP can help in both designing suitable controllers and also in analysing the regulatory mechanisms in complex systems. So far the application of IMP in biology has been case-specific and ad hoc, primarily due to the lack of generic versions of the IMP for biomolecular reaction networks that model biological processes. In this short article we highlight the need for an IMP in biology and discuss a recently developed version of it for biomolecular networks that exhibit maximal Robust Perfect Adaptation (maxRPA) by being robust to the maximum number of disturbance sources.

INDEX TERMS Biomolecular reaction networks, internal model principle, synthetic biology, systems biology.

I. INTRODUCTION

The cells that make up living organisms are an engineering marvel. They easily outperform man-made systems in terms of both the efficiency and the variety of the tasks they perform. In order for the cells to survive in a harsh and often unpredictable environment, they must ensure tight regulation of several key variables [1]. This regulation is achieved through an array of exquisitely designed biomolecular controllers that must work together coherently despite the noisy and seemingly chaotic conditions within the cells [2]. This is no easy feat, and understanding how this regulation is achieved is of fundamental importance in biology. Beyond basic science, a deeper understanding of biological regulation has strong implications for medical therapy, as the origins of many diseases have been linked to the failure of intracellular control mechanisms. Advances in quantitative measurement techniques, along with computational and mathematical tools provided by systems biology, have led to the identification and functional characterisation of many control strategies within living cells [3], [4], [5], [6], [7]. Parallel developments in synthetic biology and genetic engineering have given scientists

the unprecedented ability to engineer biomolecular controllers (e.g. using genetic components) and to deliver them inside living cells where they achieve new functions *in-vivo*. We refer to this emerging field concerned with the analysis and design of genetic control systems as *Cybergenetics* (see [8] and the references therein). Lying at the interface of control theory and systems biology, this multidisciplinary research direction offers new opportunities for engineering biology for personalized medicine, industrial biotechnology, engineered living materials, environmental remediation and sustainable agriculture, among other applications. In this article we call for systematic development of novel and broadly applicable control-theoretic principles that are specifically tailored to understanding and designing biomolecular control systems. The aim of this note is to discuss one such control-theoretic concept, the Internal Model Principle (IMP) [9], [10], [11], [12], from the perspective of biomolecular networks.

A. THE FORMALISM OF REACTION NETWORKS

Biological processes at the subcellular level can be viewed as a collection of dynamical interactions among molecular

species, such as proteins, metabolites, nucleic acids, etc., that make up the living cell. These interactions take place through chemical reactions that realize the myriad biological functions needed to sustain life. Therefore, biological processes are best modelled using the mathematical formalism of reaction networks. Consider a reaction network with N species $\mathbf{X}_1, \ldots, \mathbf{X}_N$ which participate in K reactions of the form

$$\sum_{i=1}^{N} \nu_{ik} \mathbf{X}_i \longrightarrow \sum_{i=1}^{N} \nu'_{ik} \mathbf{X}_i, \qquad k = 1, \ldots, K. \tag{1}$$
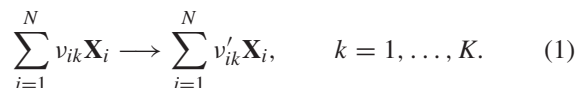
This simply says that each reaction k, consumes ν_{ik} and produces ν'_{ik} molecules of species $\mathbf{X}_i$, causing a net change of $s_{ik} := (\nu'_{ik} - \nu_{ik})$ in the molecular count of $\mathbf{X}_i$. The vector $s_k = (s_{1k}, \ldots, s_{Nk})$ is called the stoichiometric vector for reaction k and it specifies the change caused by this reaction in the molecular counts of all the species. The stoichiometric structure of this reaction network can be described by its $N \times K$ stoichiometric matrix S formed by horizontally stacking the stoichiometric (column) vectors for all the reactions. The rate of firing of each reaction k is specified by its propensity function $\lambda_k(x)$ whose argument is the vector-valued state $x = (x_1, \ldots, x_N)$ denoting the concentrations of all the N species (i.e. x_i is the concentration of species $\mathbf{X}_i$). Under the deterministic model, the state dynamics $(x(t))_{t \geq 0}$ is described by a N-dimensional system of ordinary differential equations (ODEs)

$$\frac{dx}{dt} = \sum_{k=1}^{N} \lambda_k(x) s_k.$$

In recent years it has come to light that many biomolecular species are in fact present in such low copy-numbers that the reactions involving them are random and intermittent, rather than continuous. Hence, rather than a deterministic ODE-based model, the dynamics for such systems is better captured by a stochastic continuous-time Markov chain (CTMC) model. In this CTMC $(X(t))_{t \geq 0}$ the state $X(t) = (X_1(t), \ldots, X_N(t))$ is the vector of species' copy-numbers, and its probability distribution $p(t, x) = \mathbb{P}(X(t) = x)$ (i.e. the probability of being in state x at time t) follows a high (and often infinite) dimensional system of linear ODEs called the Chemical Master Equation (CME) [13].

Typically mass-action kinetics is assumed, which stipulates that in the deterministic setting, the propensity function $\lambda_k(x)$ for the k-th reaction in (1) has the form

$$\lambda_k(x) = \theta_k \prod_{i=1}^{N} x_i^{\nu_{ik}}$$

where θ_k is the reaction rate constant. In the stochastic setting, the form of the mass-action propensity is similar, but the monomial multiplying θ_k is replaced by a combinatorial expression [13]. Mass-action reactions are generally denoted by putting the rate constant above the reaction arrow (see (1)) and this is the convention we follow in this article.

B. ROBUST PERFECT ADAPTATION NETWORKS AND THE INTERNAL MODEL PRINCIPLE

Let us consider the scenario where a reaction network comprising species $\mathbf{X}_1, \ldots, \mathbf{X}_N$ is able to tightly regulate the abundance level of the output species $\mathbf{X}_1$. This property is called *homeostasis* in biology [14], and here we assume that there is a set-point μ^* at which the output level (i.e. abundance of $\mathbf{X}_1$) is maintained, despite persistent external differences. These disturbances will certainly affect the output, but this effect would be transitory and the system would adapt by returning its output close to the set-point μ^*. In cases where the output recovers *exactly* to the set-point the system is said to be *perfectly adapting*. This notion can be further qualified by demanding that the adaptation property is maintained despite changes to the network parameters or structure, and this is referred to as Robust Perfect Adaptation (RPA). This is just the term used by systems biologists to refer to the notion of robust steady-state tracking that is familiar to control theorists and engineers. It is important to note that there are many shades of RPA, depending on the number and the type of disturbances that are rejected. We shall revisit this point a little later.

The fundamental issue that we would like to address is that of identifying the structural requirements for a network to exhibit RPA. This will enable us to understand how natural cellular processes are regulated and find the conditions which cause them to fail leading to pathological outcomes. Moreover, this structural characterisation would enable us to develop novel controller motifs that can potentially be synthetically engineered to correct for the failure of natural controllers. In this context, the Internal Model Principle (IMP) [12] from control theory provides a clue on how RPA systems may be organised. This principle asserts that for a system to be robust to a class of dynamic disturbances, it must contain a subsystem, called the *internal model* (IM), that is able to generate these disturbances, and then pass counteracting signals to the *rest of the network* (RoN) in a feedback fashion (see Fig. 1). Moreover, the IM only has access to one signal, which is the regulated output variable (i.e. $x_1(t)$ which is the concentration of $\mathbf{X}_1$) and it does not have access to the other internal states of the system or even the disturbance it seeks to recreate. In this article, we shall only consider constant-in-time disturbances, in which case the IM must implement an "integrator" in the sense that it must be able to generate the time-integral of the deviation of the output $x_1(t)$ from its set-point μ^*. This gives rise to the familiar integral feedback mechanism in control theory which is a workhorse in modern control technology. We must mention that IMP is not a single mathematical result, but rather it provides a template for several possible results, each of which must be independently proved under their specific assumptions about the underlying system and the disturbances. Thus far, general forms of IMP are available for linear systems [9], [10] and nonlinear systems with linear effect of disturbances [11] i.e. the system's dynamics is
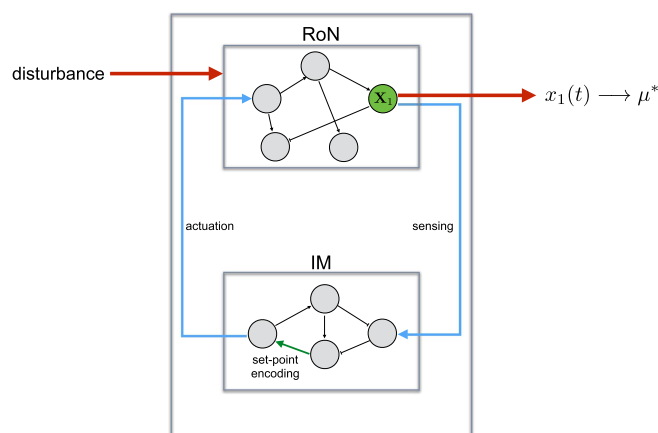
(a)

(b)
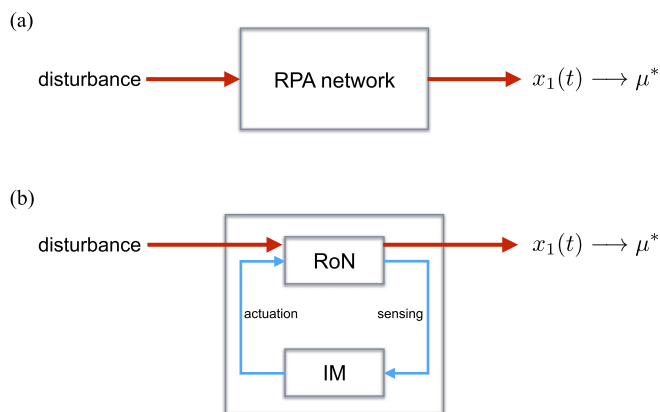
FIGURE 1. Consider a RPA network that at steady-state is able to maintain the output $x_1(t)$ (concentration of X_1) at the set-point μ^*, despite being affected by disturbances that modify the network's structure or parameters (panel (a)). The Internal Model Principle (IMP) states that such a system can be decomposed (possibly after a change of coordinates) into an *internal model* (IM) and the *rest of the network* (RoN), as shown in panel (b). The IM estimates the effect of disturbances by *sensing* the output, measuring its deviation from the set-point μ^*. It then computes the time-integral of this deviation and uses this signal to *actuate* the RoN by passing it as input.

FIGURE 2. This figure shows a biomolecular version of the IMP for RPA networks, where the IM and the RoN comprise of biomolecular species and reactions. Moreover sensing and actuation are also carried out by such reactions. The set-point μ^* is a function of the parameters describing the kinetics of these reactions, as well as additional set-point encoding reactions that reside within the IM module. Only one such set-point reaction is shown but there can be many.

given by

$$\frac{dx}{dt} = f(x) + ug(x),$$

where f and g are nonlinear functions of the state variable x, and u is the disturbance input which enters linearly into the dynamics. For a recent review on IMP, we refer the readers to [12].

Generally, biomolecular networks exhibiting RPA are nonlinear and even the disturbances can enter their dynamics nonlinearly, typically via perturbations of the reaction propensities. To the best of our knowledge, there do not exist versions of IMP for such nonlinear systems, and hence it remains unclear if the implementation of an integrator is even necessary for RPA. Furthermore, existing IMP results are mostly *analytical* rather than *mechanistic*, i.e. the decomposition of a RPA system into an IM and a RoN (as shown in Fig. 1) holds under a nonlinear change of coordinates which can be analytically determined by solving certain partial differential equations (PDEs). While this approach works for many systems, it inhibits the physical interpretability of the IM, which implements the controller in terms of biomolecular species and reactions. Indeed, in the transformed coordinate system the state-variables no longer correspond to species' abundances and in fact they may not even be positive. Implementation of integral control with only positive variables is a unique feature of biomolecular RPA systems which makes it distinct from the prevalent control techniques in man-made systems. It is self-evident that having a physically meaningful and biologically implementable IM is crucial for synthetic biology applications. The issues mentioned above suggest the need to develop a biomolecular version of the IMP, where a

RPA network naturally decomposes into an IM and a RoN without the need of any nonlinear coordinate-transformations. As shown in Fig. 2 the IM must itself be a subnetwork, consisting of biomolecular species and reactions, which implements the integrator for the difference between the output species concentration $x_1(t)$ and the set-point μ^*. Here actuation and sensing are also biomolecular reactions and the set-point μ^* is encoded by the parameters (e.g. rate constants) describing the kinetics of these reactions or the IM reactions. While the general form of such an IMP remains elusive, in Section III we discuss a recently developed version of a biomolecular IMP where the class of disturbances to be rejected is *maximal*. Interestingly, this result has been developed for both deterministic and stochastic scenarios, and it shows how the presence of randomness in the dynamics restricts the structure of the IM.

II. EXAMPLES OF BIOMOLECULAR RPA NETWORKS
We now discuss three known examples of biomolecular RPA networks and connect them to the IMP. In all the examples we shall assume mass-action kinetics.

A. THE ANTITHETIC CONTROLLER
In Fig. 3(a) we show an arbitrary network with output species X_1 being regulated by the *antithetic controller* [15], that consists of two species X_2 and X_3 that annihilate each other via a reaction of the form $X_2 + X_3 \longrightarrow \emptyset$.[1] Here the sensing reaction is $X_1 \longrightarrow X_1 + X_3$ with rate constant θ_2, and there is a single set-point encoding reaction $\emptyset \longrightarrow X_2$ with the rate constant θ_1. The ratio of these two rate constants encodes the

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[1] Here $\emptyset$ denotes the null species whose abundance is assumed to be a constant and so its dynamics is not considered. It can also refer to an inactive complex which does not influence the modelled reaction network.
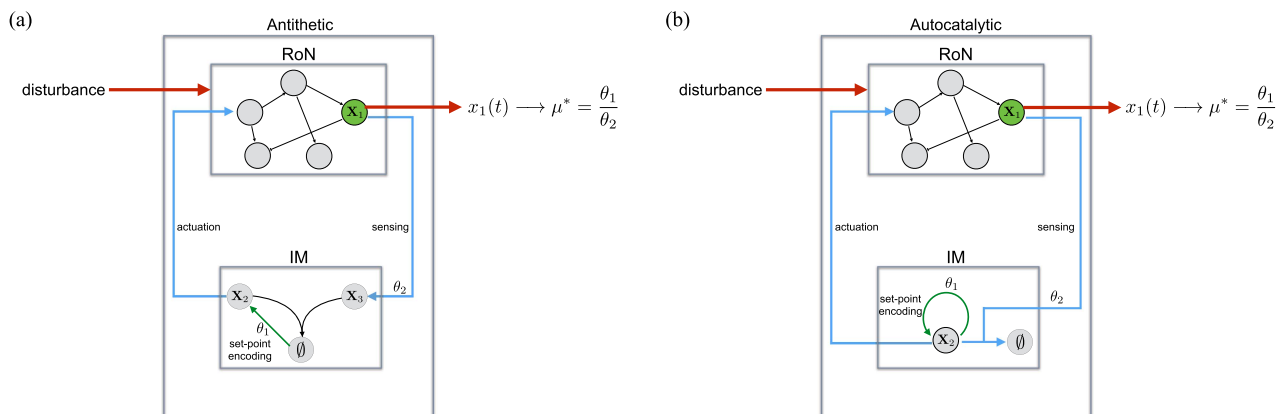
FIGURE 3. This figure depicts two synthetic controllers (antithetic [15] and autocatalytic [16]) that regulate the concentration of an output species X_1 in an arbitrary intracellular network. The biomolecular controller forms the internal model (IM) while the regulated network becomes the rest of the network (RoN). In both the cases, the set-point μ^* is the ratio between the rate constant θ_1 of the solitary set-point encoding reaction within the IM and the rate constant θ_2 of the sensing reaction.
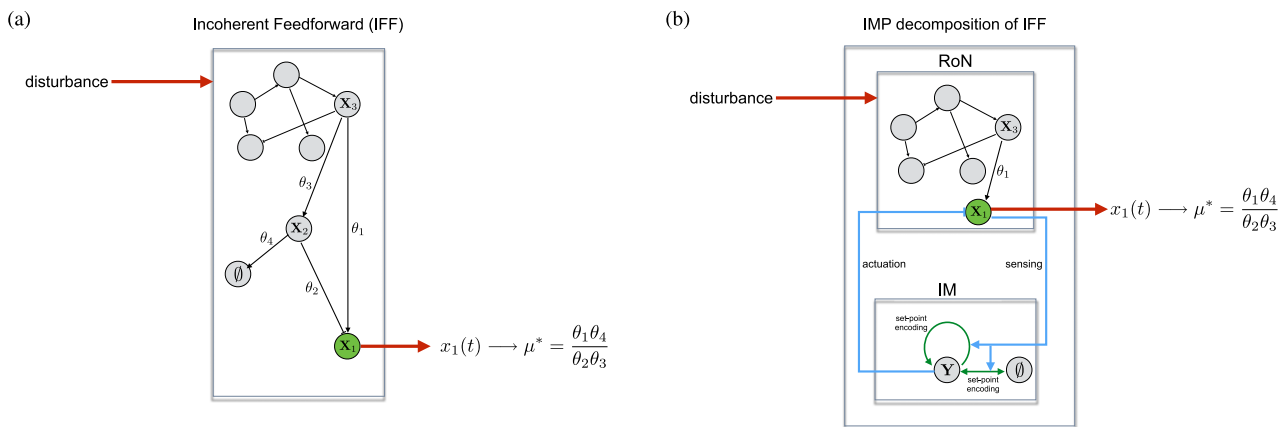
FIGURE 4. This figure presents an example of an incoherent feedforward (IFF) network (panel (a)) and its IMP-based decomposition (panel (b)). In such a network there are two branches from the input species X_3 to the output species X_1, with opposite effects. The direct branch promotes the production of X_1 while the indirect branch contains an inhibitor species X_2 which catalyses the degradation of X_1. The set-point is determined by a ratio of the rate constants of the four reactions present in the two input-output branches, and hence it is insensitive to the disturbances that affect the input node X_3. In panel (b) we depict the IMP-based decomposition of the IFF network, with the IM containing a fictitious species Y defined by (4). The reactions in this figure have non-standard kinetics that mimic the ODE system (5). Moreover, there are multiple set-point encoding reactions, unlike the previous examples.

set-point μ^* and species X_2 actuates the regulated network by catalytically inducing the production/degradation of some internal species in RoN which is connected to the output X_1.

B. THE AUTOCATALYTIC CONTROLLER
In Fig. 3(b), the setting is similar, but now the regulator is the *autocatalytic controller* [16] with a single species X_2 that stimulates its own production via the set-point encoding reaction $X_2 \longrightarrow 2X_2$ with rate constant θ_1. Again the set-point is the ratio of θ_1 and the rate constant θ_2 of the sensing reaction $X_1 + X_2 \longrightarrow \emptyset$. This form of the set-point shows that in both the above examples, the output species is robust to disturbances that perturb the parameters/structure of the regulated network. Achieving this robustness was the motivation behind the design of these synthetic controllers, and here the IMP-based decomposition into the IM (controller) and the RoN (the regulated network) is by design. We now present

another example where this IMP-based decomposition is not straightforward.

C. THE INCOHERENT FEEDFORWARD NETWORK
Rather than having a natural feedback structure, many endogenous RPA networks have an incoherent feedforward (IFF) structure [17], which is characterised by two branches, with opposite effects from the input node (entry point of disturbances) to the output. Adaptation arises due to cancellation of these effects under certain conditions. Examples of IFF-based adaptation circuits include, EGF to ERK activation [18], nitric oxide to NF-κB stimulation [19], microRNA regulation [20] etc. In Fig. 4(a) we show a IFF network which models a sniffer system [21]. Here the disturbances enter via the input species X_3 which both positively and negatively affects the output species X_1. While the positive effect is direct, the negative effect is via the production of the inhibitor species

$\mathbf{X}_2$ that catalyses the degradation of $\mathbf{X}_1$. Let $x_1(t)$ and $x_2(t)$ be the concentrations of $\mathbf{X}_1$ and $\mathbf{X}_2$ respectively. The ODEs for their dynamics are given by

$$\dot{x}_1(t) = \theta_1 x_3(t) - \theta_2 x_1(t) x_2(t)$$
$$\dot{x}_2(t) = \theta_3 x_3(t) - \theta_4 x_2(t). \tag{2}$$

It is immediate that if the dynamics is stable then

$$\lim_{t \to \infty} x_1(t) = \mu^* = \frac{\theta_1 \theta_4}{\theta_2 \theta_3} \tag{3}$$

and hence the steady-state output is robust to disturbances that affect the dynamics of the input species $\mathbf{X}_3$. In order to find an IMP-based decomposition of this RPA network we must substitute $\mathbf{X}_2$ with a fictitious species defined as

$$\mathbf{Y} := \frac{\theta_1}{\theta_3} \mathbf{X}_2 - \mathbf{X}_1. \tag{4}$$

With this change in coordinates, we can transform the system (2) into

$$\dot{x}_1(t) = \theta_1 x_3(t) - \frac{\theta_2 \theta_3}{\theta_1} x_1(t)(x_1(t) + y(t))$$
$$\dot{y}(t) = \frac{\theta_2 \theta_3}{\theta_1} (x_1(t) + y(t))(x_1(t) - \mu^*) \tag{5}$$

where $y(t)$ is the concentration of the fictitious species $\mathbf{Y}$ and μ^* is the set-point (3). Observe that unlike $x_2(t)$, the dynamics of $y(t)$ does not have direct dependence on $x_3(t)$. Instead, its production is now promoted by the output species $\mathbf{X}_1$ and it negatively affects the concentration of $\mathbf{X}_1$. This gives rise to the feedback structure, along with separation into IM and RoN, as mandated by the IMP (see Fig. 4(b)). Observe that the time-derivative $\dot{y}(t)$ is proportional to the error signal $e(t) = x_1(t) - \mu^*$, and hence $y(t)$ behaves like an integrator for the system. Consequently, $y(t)$ can be negative and so it cannot be interpreted as the concentration of a real biological species.

In the IFF example the set-point μ^* is sensitive to four parameters, while in the examples considered in Fig. 3, the set-point is only sensitive to two parameters. Therefore IFF possesses a lesser degree of robustness in comparison to the examples in Fig. 3. To elaborate this point further, let us reconsider this IFF example under the scenario that the output species $\mathbf{X}_1$, in addition to being actively degraded by species $\mathbf{X}_2$, also degrades independently via the reaction $\mathbf{X}_1 \longrightarrow \emptyset$ with rate constant θ_5. In this case, the ODE for $x_1(t)$ becomes

$$\dot{x}_1(t) = \theta_1 x_3(t) - \theta_2 x_1(t) x_2(t) - \theta_5 x_1(t),$$

and the steady-state output changes to

$$\lim_{t \to \infty} x_1(t) = \frac{\theta_1 \theta_4 x_3^*}{\theta_2 \theta_3 x_3^* + \theta_4 \theta_5},$$

where x_3^* is the steady-state value of the concentration of the input species $\mathbf{X}_3$. This shows that the output is no longer robust to the disturbances that affect the input x_3^*. The reason for this is that the balancing mechanism between the two IFF

branches fails — as $\mathbf{X}_2$ cannot take the additional loss of $\mathbf{X}_1$ molecules into account (due to the feedforward structure), it overcompensates for the positive effect of the input $\mathbf{X}_3$ on $\mathbf{X}_1$, leading to the loss of RPA property.

Such problems do not arise for the examples in Fig. 3 because they exhibit *maximal Robust Perfect Adaptation* (maxRPA) as their set-points depend on only two parameters (unlike IFF). As we next discuss, a maxRPA network is naturally endowed with an IMP-based feedback structure and its integral mechanism is naturally encoded by biomolecular species and reactions. This should be contrasted with the IFF example, where the IMP-based decomposition seems contrived as it requires us to define non-physical species and interactions.

III. IMP FOR maxRPA NETWORKS
In this section, we present the generic structural requirements for maxRPA networks, such as those in Fig. 3, and argue that they have an IMP-based decomposition and their IM (and RoN) can be easily identified using certain linear algebraic conditions, in both deterministic and stochastic settings. These results are presented informally, but their precise mathematical statements, along with the proofs, can be found in [22].

Suppose that output set-point μ^* is some function of the rate constants $\theta_1, \ldots, \theta_m$ of m network reactions with mass-action kinetics. For maximal robustness we must have m as small as possible, and it can be shown that the least non-trivial value is $m = 2$ and in this case μ^* is some function of the ratio θ_1/θ_2 of two rate constants [22]. Suppose a network consists of N species (viz. $\mathbf{X}_1, \ldots, \mathbf{X}_N$) and K reactions. By rearranging the reactions if necessary, we can assume that the first two reactions have mass-action kinetics with rate constants θ_1 and θ_2 respectively, while the kinetics of the other reactions is arbitrary and depends on parameters which can be altered by disturbances. These disturbances are rich enough to independently alter the kinetics of each reaction and we assume that the overall network dynamics is stable. As mentioned before, the structure of this network is specified by a $N \times K$ stoichiometric matrix whose columns are the stoichiometric vectors for each of the reactions. Typically $K > N$ and we shall assume that S has full row-rank N. Under these conditions the network exhibits maxRPA in the deterministic setting, if and only if the following two conditions are met

1) The first two reactions have as reactants,[2] unequal number of molecules of $\mathbf{X}_1$ but equal number of molecules of the other species $\mathbf{X}_2, \ldots, \mathbf{X}_N$.
2) There is a N dimensional row vector $q = (q_1, \ldots, q_N)$ and a positive number κ such that

$$qS = (\kappa, -1, 0, \ldots, 0). \tag{6}$$

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[2] The term reactant refers to species on the left hand side of the reaction arrow. For example, a reaction of the form $2\mathbf{X}_1 + \mathbf{X}_2 \longrightarrow *$ has two reactants $\mathbf{X}_1$ and $\mathbf{X}_2$ with molecules 2 and 1 respectively.

This result is proved in [22] where an integrator for the dynamics is also constructed. The set-point for the output is given by

$$\mu^* = \left(\kappa \frac{\theta_1}{\theta_2} \right)^{\frac{1}{\alpha}},$$

where α is the number of reacting $\mathbf{X}_1$ molecules in the second reaction minus those in the first reaction. Under our conditions the pair (q, κ) satisfying (6) is unique, if it exists.

Let us see how these conditions apply to the maxRPA networks considered in Fig. 3. For the antithetic network, the first two reactions are $\emptyset \rightarrow \mathbf{X}_2$ and $\mathbf{X}_1 \rightarrow \mathbf{X}_1 + \mathbf{X}_3$. Clearly the first condition is satisfied and since $\alpha = 1$, the set-point is just the ratio θ_1/θ_2 as mentioned before. To see how the second condition is satisfied note that there are only three reactions that change the molecular counts of $\mathbf{X}_2$ and $\mathbf{X}_3$. These include the first two reactions with stoichiometric vectors $\zeta_1 = (0, 1, 0, \ldots, 0)$ and $\zeta_2 = (0, 0, 1, 0, \ldots, 0)$ respectively, and the only IM reaction which is the annihilation reaction $\mathbf{X}_2 + \mathbf{X}_3 \longrightarrow \emptyset$ with stoichiometric vector $\zeta_3 = (0, -1, -1, \ldots, 0)$. Therefore if we set $q = (0, 1, -1, 0, \ldots, 0)$ and $\kappa = 1$ then (6) is satisfied, and this implies that the antithetic network is maxRPA. Similarly, we can check that these conditions are satisfied for the autocatalytic network in Fig. 3(b). In this example, the first two reactions are $\mathbf{X}_2 \longrightarrow 2\mathbf{X}_2$ and $\mathbf{X}_1 + \mathbf{X}_2 \longrightarrow \emptyset$ with stoichiometric vectors $\zeta_1 = (0, 1, 0, \ldots, 0)$ and $\zeta_2 = (-1, -1, 0, \ldots, 0)$ respectively, and there are no other reactions that modify $\mathbf{X}_2$. It is immediate that the first condition is fulfilled and the second condition (6) also holds for $q = (0, 1, 0, \ldots, 0)$ and $\kappa = 1$.

RPA networks can be classified as either *homothetic* or *antithetic* based on the signs of non-zero components in the vector q that satisfies (6). Such a network is called *homothetic* if all the components have the same sign, and otherwise it is called *antithetic*. The example in Fig. 3(a) is a minimal[3] example of an antithetic RPA network, while the autocatalytic network in Fig. 3(b) (along with zero-order degradation networks [6]) is a minimal homothetic network (see [22] for more details). Antithetic RPA networks are characterised by the presence of an annihilation reaction between two IM species.

We now briefly discuss the stochastic setting where the dynamics is given by a CTMC and the output of interest is the expected copy-number of the output species $\mathbf{X}_1$ at the steady-state. It can be shown that maxRPA characterising conditions become more restrictive in this setting in comparison to the deterministic scenario (see [22]). While the second linear-algebraic condition (6) is the same, the stoichiometric requirements for the first two reactions become more stringent. These two reactions cannot have species $\mathbf{X}_2, \ldots, \mathbf{X}_N$ as reactants, and only one of these two reactions should have exactly one molecule of the output species $\mathbf{X}_1$ as reactant, while the other should have none. Therefore while the network

in Fig. 3(a) exhibits maxRPA in the stochastic setting, the network in Fig. 3(b) does not.

We now discuss how the vector q that satisfies (6) allows us to come up with a IMP-based decomposition of a maxRPA network, as shown in Fig. 2. The internal model (IM) will consist of all those species for which the corresponding component in the q vector is non-zero, while all the other species belong to the rest of the network (RoN). Under the natural assumption that disturbances that enter via the last $(K - 2)$ reactions can affect the output species, it can be shown that $q_1 = 0$, and so $\mathbf{X}_1$ belongs to RoN as in Fig. 2. According to our maxRPA characterisation conditions, the first two reactions must have unequal number of molecules of $\mathbf{X}_1$ as reactants. We call the reaction with the highest number of such reactions as the *sensing* reaction while the other is the *set-point encoding* reaction. This completes the picture in Fig. 2 for maxRPA networks. Note that while our conditions are silent on the form of actuation reactions, their presence is necessary for the stability of the overall system.

One interesting consequence of this IMP is that any maxRPA network in the stochastic setting must necessarily be antithetic. This is because if a network is homothetic, all the non-zero components of q have the same sign. Then the first two components on the right hand side of (6) cannot have opposite signs because the species in the IM cannot be reactants in the first two reactions, and so the corresponding components in the first two columns of S can only be non-negative. In fact, all stochastic maxRPA networks must have a generalised antithetic topology that embeds the minimal antithetic motif in Fig. 3(a) (see [22] and [23]).

IV. CONCLUSION

The celebrated internal model principle (IMP) provides a necessary condition for asymptotic regulation of a dynamical system that is subject to disturbances. Specifically, IMP asserts that this regulation can only be achieved if the system embeds a subsystem that controls by using feedback of the regulated variable and is able to reduplicate the dynamic structure of the disturbances. While murmurings of such a principle can be found in early texts on psychology and the human nervous system [24], IMP was first mathematically formalised by Francis and Wonham [9] for linear systems. Since then IMP has enjoyed a rich history of development and generalisation (see [12] for a recent review), and it is now a centrepiece in modern control theory. It is instructive to think of the IMP, not as a single result but rather as a mold for generating many possible results, depending on the scientific area and the specific mathematical assumptions. Once established, IMP serves as a dual-purpose tool – it can facilitate design of control systems and it can also be used to analyse complex systems to understand their regulatory mechanisms. In the context of biology, the former would be a synthetic biology perspective while the latter would be that of systems biology. While the IMP has been applied to these biological domains [12], there do not exist general versions

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[3]Here by minimality we mean that the IM has the least number of species and reactions (in this order). Minimal motifs may not be unique.

of the IMP that are specifically tailored for biomolecular reactions networks that model intracellular processes. Our goal in this article was firstly to provide a conception of IMP for biomolecular networks, and secondly to present a recently developed version of this IMP for networks exhibiting maximal Robust Perfect Adaptation (maxRPA) [22]. This result shows that maxRPA networks naturally possess an embedded controller, comprising biomolecular species and reactions, that performs the necessary integral action in a feedback fashion. On the other hand, networks that exhibit weaker forms of RPA (like the incoherent feedforward (IFF) circuit [21]) may not have such a natural integral feedback structure, and in order to cast them into the IMP framework, use of non-physical species and reactions could be needed.

In this article we only focused on biomolecular networks that perfectly adapt to constant-in-time disturbances that enter via parameter or network variations. We call upon the research community to generalise IMP to *imperfectly* adapting systems and also for more complicated dynamic disturbances (e.g. oscillations). Indeed, many biological network exhibit imperfect adaptation [25] and delineating these mechanisms would help understand the trade-offs related to perfect adaptation, and identify scenarios where the associated costs outweigh the benefits of tight regulation. Similarly, the environment of biological cells is often rife with periodic oscillatory signals (e.g. ligand concentrations [26]), and in certain applications it may be important to faithfully track these signals within the cells. In such scenarios, versions of the biomolecular IMP for oscillatory signals would greatly help in the analysis and design of intracellular controller mechanisms that can robustly reduplicate such signals despite the tortuous complexity of the intracellular milieu.

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