Absolutely Robust Controllers for Chemical Reaction Networks

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Abstract

In this work, we design a type of controller that consists of adding a specific set of reactions to an existing chemical reaction network in order to control a target species. This set of reactions is effective for both deterministic and stochastic networks, in the latter case controlling the mean as well as the variance of the target species. We employ a type of network property called absolute concentration robustness (ACR). We provide applications to the control of a multisite phosphorylation model as well as a receptor-ligand signaling system.

For this framework, we use the so-called deficiency zero theorem from chemical reaction network theory as well as multiscaling model reduction methods. We show that the target species has approximately Poisson distribution with the desired mean. We further show that ACR controllers can bring robust perfect adaptation to a target species and are complementary to a recently introduced antithetic feedback controller used for stochastic chemical reactions.

Keywords absolute concentration robustness, control, reaction networks, Possion distribution, multiscaling, deficiency zero.

1 Introduction

In this paper we propose a set of synthetic controllers that can be added to a given chemical reaction network in order to control the concentration or copy number of a given species of interest. Chemical reaction networks describe a variety of problems in engineering and biology, and there has recently been a surge in interest for stochastic models of such networks [1–4]. Stochastic effects are important in order to describe the noise inherent in reactions with low numbers of molecules, as is often the case inside individual cells. The techniques proposed in this paper will be shown to apply both in the deterministic case, where concentrations are described by ordinary differential equations, as well as in the stochastic case where the dynamics are described by a discrete time, continuous space Markov process.

The controllers used in our framework are inspired by a property called *absolute concentration robustness* (ACR). We provide both a theoretical framework and computational simulations in several specific biochemical systems to show that an ACR controller can shift all positive steady state values of a target species towards a desired value. We also show that in stochastic networks, the ACR controller can account for the intrinsic noise in the chemical reaction. We approximate the

behaviour of the target species using a reduced chemical reaction model derived through multiscaling analysis. Our stochastic analysis assumes certain conditions on the topology of the controlled network that are described using the so-called *deficiency* of the system. These two theoretical tools will be combined to calculate the behaviour of the reduced system, as well as to show that the behaviour of the target species in the reduced system approximates that of the original network. Using computational simulations we also explore the *robust perfect adaptation* of the target species in the controlled system, a highly desirable goal in control theory.

When a dynamical system has multistationarity or the dynamics are confined to a lower-dimensional subset by conservation relations among species, the long term behaviour depends on the initial conditions of the system. In general different initial concentrations may lead to different long term steady states of the different species. However sometimes the positive steady state values of a species of interest are identical independent of the initial conditions. Such a system is said to possess the ACR property as the steady state of the dynamics is robust to the initial conditions. In that case, the species with identical steady state values is called the ACR species. This counter-intuitive dynamical aspect was proposed by Shinar and Feinberg in 2010 [5], where they further provided network topological conditions ensuring that the associated deterministic system admits ACR.

For a simple example of an ACR system consider the following network, which will be the basic ACR controller throughout this manuscript,

$$Z + A \xrightarrow{\theta} 2Z, \quad Z \xrightarrow{\mu} A. \tag{1}$$

Both reactions produce and consume the same amount of A and Z, hence the total amount of $Z+A$ is conserved. One can think of A and Z as being different forms of the same protein, say active and inactive. Let $a(t)$ and $z(t)$ be the concentration of the species A and Z, respectively. Assuming the associated dynamical system is equipped with mass-action kinetics [6], the concentration of Z follows the equation

$$\frac{d}{dt}z(t) = \theta a(t)z(t) - \mu z(t). \tag{2}$$

At steady state one can set the right hand side equal to zero, and assuming $z \neq 0$ one obtains that the steady state value for A is $\frac{\mu}{\theta}$. Letting $a(0) + z(0) = N$ be the initial input of the system, the only positive roots of the right-hand side of (2) are $(a, z) = (\frac{\mu}{\theta}, N - \frac{\mu}{\theta})$. Hence this system is an ACR system and species A is an ACR species. See Figure 1b for a phase plane diagram illustrating this behaviour.

In comparison to the ACR network in (1), we consider the simple reaction

$$A \underset{4}{\overset{2}{\rightleftharpoons}} B, \tag{3}$$

where the total mass of A and B is also invariant in time. In both systems the dynamics is confined to one of the black straight lines in Figure 1a and 1b. The positive steady states of this system lie on the intersections between the nullclines (red) and the phase planes (black). Hence for system (3), the steady state values of both A and B vary depending on the initial condition as shown in Figure 1a. On the other hand, the ACR network system (1) is such that all the positive steady states for A are identical, as shown in Figure 1b.

A similar type of control has been considered by Mustafa Khammash and others in Briat et. al. [7]. In that work, Khammash and colleagues propose an antithetic integral feedback circuit

$$Z_1 + Z_2 \xrightarrow{\eta} 0 \xrightarrow{\mu} Z_1 \xrightarrow{k} Z_1 + X_1, \quad X_\ell \xrightarrow{\theta} X_\ell + Z_2,$$

that robustly stabilizes a species of interest in the presence of intrinsic noise. In the controlled system the mean of the stochastic dynamics of a target species is stabilized at a pre-specified value with a low metabolic cost. A recent follow-up work [8] experimentally implemented the antithetic control circuit in a growth-rate control system in *E. coli*. We point out that this antithetic control circuit satisfies the topological conditions for ACR provided by Shinar and Feinberg in [5], and thus it is a specific example of an ACR system. In particular, the control species Z_1 and Z_2 are ACR species if the system admits a positive steady state. There are however a few important differences with our work. As we will show, the ACR controllers aim to control the mean, variance and even higher moments of a target species by controlling its distribution. The controllers in [7, 8] are designed to robustly control the mean of the target species, but the controller might increase its noise. To account for the noise in the target species, Briat et. al. show in the follow-up work [9] that for a unimolecular model, an additional negative feedback loop can reduce the noise up to the original variance. Also, stochastic ACR controllers control the target distribution approximately assuming that sufficient copies of the control species present, while the control proposed by [7, 8] provides exact control without approximation scheme.

A particularly powerful property of an ACR system is that it can endow the ACR property to a given network. When an ACR system is added to a non-ACR model, one of the species in the combined system could become absolutely robust. For example, suppose that species A is present in a given deterministic network but that Z is not, and that we add the two reactions (1). Then the dynamics of Z will still satisfy $z(t) = \theta az - \mu z$. Moreover, at steady state it must still hold $a = \mu/\theta$ due to the same analysis as before. Thus species A is now absolutely robust in the new system.

If the ACR property of the ACR system is inherited to the target species in the controlled system, then we call the ACR system an *ACR controller*. Throughout this paper we also call the newly introduced species in the ACR controller a *control species*. In the following sections, we show that the steady state value of the target species is tunable with the parameters of the ACR controller. In the Supplementary Material, we further investigate the local stability of the steady state in the controlled system.

While the ACR controller has the ability to control a target species in deterministic systems, chemical species are often modeled as discrete entities. Stochastic models in biology have become increasingly relevant, as people have noticed that intrinsic noise significantly contributes to the dynamical behaviour [10–15]. The effects of noise are especially large if the abundance of a species in the system is low. Many important biochemical models consist of species with low copy numbers inside each individual cell [4]. More details on the modeling of stochastic networks are included in the Supplementary Material. In stochastic models we have additional control goals than for deterministic models, as it is important to not only control the mean expression level but also its variance (i.e. noise) and ideally the full probability distribution of the target species. A room that varies in temperature between 0° C and 50° C might be said to be controlled with mean 25° C, though its occupants would probably disagree.

In order to control stochastic systems, we rely on the mathematical theory of deficiency in chemical reaction networks. The *deficiency* of a reaction network is a non-negative integer that

is determined by the topology of the network regardless of parameter values. Networks with deficiency zero and a weak reversibility property have well characterized long term dynamics, under both deterministic and stochastic conditions. In the deterministic case, such systems admit a unique local asymptotically stable steady state for given total amounts of the species [16–18]. For a stochastic system, under the same conditions, each of the species has Poisson distribution centered around its deterministic steady state [1] (see the Supplementary Material for additional details). These strong properties inspire us to propose a new deficiency based control scheme for stochastic reaction networks, based on recent work expanding ACR to the stochastic case [19, 20].

One property observed in some stochastic chemical reaction networks is a so-called *extinction event*. Such an event takes place when some of the species disappear and can never return to the system. A stochastically modeled ACR controller can go extinct if a control species, such as Z in the basic ACR controller, is entirely removed from the system. This phenomenon is commonly present in ACR networks [21]. One way to minimize this effect is to run the controllers with sufficiently high control species abundance, so that a potential breakdown of the ACR system is rare.

This high abundance setting is indeed a suitable assumption for the study of stochastic systems [22]. In our stochastic systems, each species can be categorized as either high abundance or low abundance, compared with the total protein abundance N. We use N as a scaling parameter, and we carry out a multiscaling procedure to reach a reduced stochastic reaction network. By assuming that the reduced network has zero deficiency and is weakly reversible, we conclude that the target species has approximately Poisson distribution both in the reduced and the original networks. In special cases, the reduced model can be treated as a hybrid between deterministic and stochastic networks [20, 23, 24].

We provide multiple examples of control of given biochemical networks using different ACR controllers. For example, we use an existing deterministic model of ERK signal transduction from Rubinstein et al [25], and we stabilize the dose response of this system using the basic ACR controller. We also study a stochastic receptor-ligand model in which we target the concentration of free receptor, and we show that the concentration of a downstream regulatory protein is also controlled as a result. Finally, we study a stochastic dimer-catalyzer model together with an expanded ACR controller as an application of the hybrid approach. Simulations using the Gillespie algorithm are provided throughout to illustrate the control implemented by our approach. We also emphasize that the controlled networks admit a robust perfect adaptation property for both deterministic and stochastic examples.

2 Results

2.1 Deterministic Control Using ACR Networks

We begin by using an ACR controller for a deterministic system. Consider again the simple translation model between species A and B,

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B. \tag{4}$$

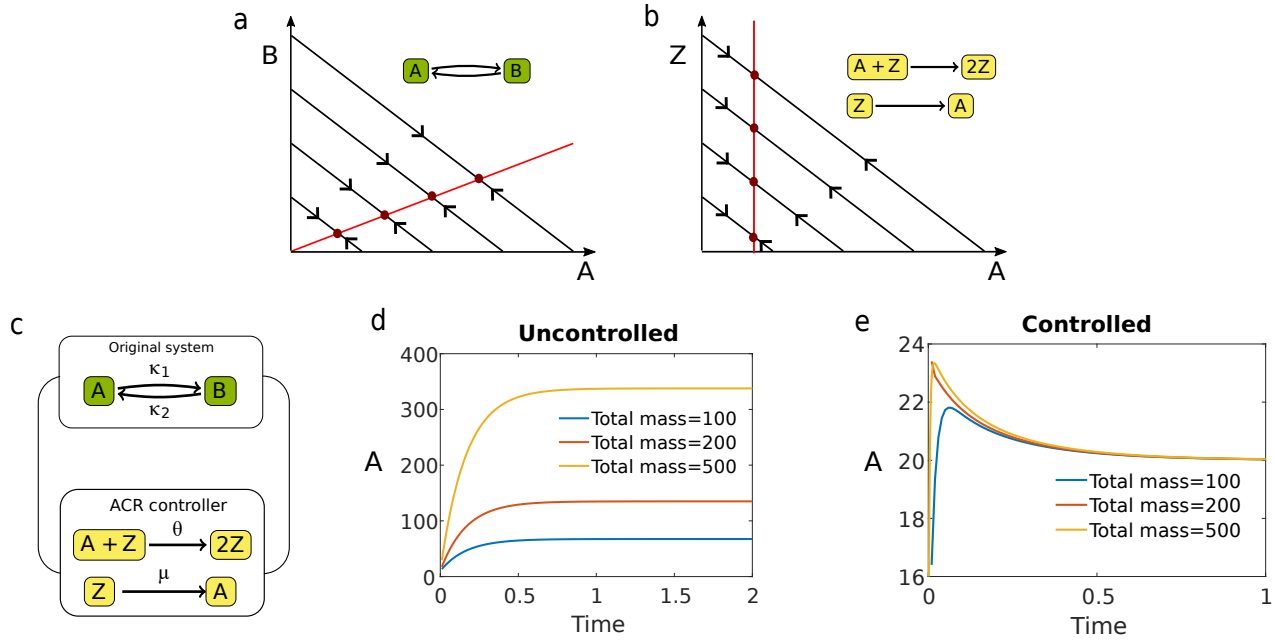

Figure 1: **a.** and **b.** Dynamics of the networks $A \rightleftharpoons B$ and the basic ACR controller, respectively. The intersection between each black line and the red line is a steady state for a given total mass. **c.** The original model in 1a is controlled using the basic ACR controller. **d.** Time evolution of A in the original system, $k_1 = 2, k_2 = 4$. **e.** Time evolution of A in the controlled system via the ACR controller, using the above parameters and $\theta = 1$, $\mu = 20$.

Letting $a(t)$ and $b(t)$ denote the concentration of species A and B, respectively, the associated deterministic system with mass-action kinetics is

$$\frac{d}{dt}a(t) = -k_1 a(t) + k_2 b(t), \quad \frac{d}{dt}b(t) = k_1 a(t) - k_2 b(t). \tag{5}$$

We notice that $\frac{d}{dt}a(t) + \frac{d}{dt}b(t) = 0$ which implies that the total mass $a(t) + b(t)$ is conserved. When $a(0) + b(0) = N$, the steady state of the system is $(a^*, b^*) = (\frac{\kappa_2}{\kappa_1+\kappa_2}N, \frac{\kappa_1}{\kappa_1+\kappa_2}N)$ by using the conservation $a(t) + b(t) = N$. Hence the positive steady state concentration of A in the original system (4) varies along with the initial input N. To get the desired steady state value for A, therefore, fine-tuning of the initial condition N is necessary.

Adding the basic ACR controller (1) to the original system, we have a new system

$$Z + A \xrightarrow{\theta} 2Z, \quad Z \xrightarrow{\mu} A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B. \tag{6}$$

As described in the introduction, using the equation for Z we can deduce that for any positive steady state it must hold $a^* = \frac{\mu}{\theta}$. See Theorem 6.2 in the Supplementary Material for a generalization of this statement to other networks as well as systems with reaction kinetics different from mass action.

As an application, we use the basic ACR network to control a system of an extracellular signal regulated kinase (ERK) activation shown in Rubinstein et al [25]. ERK is a widely studied protein in signal transduction, and it is activated through phosphorylation at two different sites. The steps

of the dual phosphorylation are regulated by other protein kinases [25]. We denote ERK by S, and consider the four phosphorylation forms S_{00}, S_{01}, S_{10} and S_{11} depending on the phosphorylated sites. Nonsequential ERK phosphorylation is mediated by mitogen-activated protein kinase MEK, denoted here by E. The variable F denotes a nonspecific phosphatase that mediates ERK dephosphorylation. The steps of phosphorylation and dephosphorylation are described with the reaction network model in Figure 2a.

In the ERK system in Figure 2a, there are three conservation relations. For instance, $E_{tot} = E + [ES_{00}] + [ES_{01}] + [ES_{10}]$ represents the total concentration of kinase, and similarly for total substrate S_{tot} and total phosphatase F_{tot}. It has been shown that the mass action deterministic model associated with the ERK model in Figure 2 a has different long-term dynamical behaviour depending on the system parameters [25–27]. These dynamical behaviours include unique stable stationarity, sustained oscillations, and bistability. We use the parameters in Rubinstein et al., which are such that the ERK system converges to a unique, stable, and positive steady state [25].

One of the most important features of this system is its so-called dose response, which describes active ERK S_{11} as a function of the kinase intput E. However this dose response depends on the total amount of phosphatase F_{tot}. We introduce a control using the basic ACR controller in order to fix S_{11} for every given value of total kinase E_{tot}, and therefore to stabilize the dose response. As the plot on the right-hand side of Figure 2b shows, the steady state concentration of protein S_{11} is sigmoidal as a function of E_{tot} for fixed F_{tot}. The goal of control with the basic ACR system in Figure 2a is to equalize the positive steady state of the phophatase F for any F_{tot}, and eventually to obtain the same sigmoidal curve for the steady state concentration of S_{11}, see Figure 2c.

Aside from the mathematical model, it is important to think how the basic ACR system in Figure 2a could be implemented experimentally. There are several possible approaches which might depend on the individual system. In this case, suppose that the phosphatase F is bifunctional, acting as a phosphatase in its standard form and turning into a kinase Z when it is itself phosphorylated. Suppose that kinase Z mediates the phosphorylation of protein F as depicted with the reaction $F + Z \to 2Z$. Finally, another phosphatase, which is not explicitly modeled in this system, eventually dephosphorylates Z into F as described with the reaction $Z \to F$. This set of assumptions would suffice to implement the control network. Notice that bifunctional enzymes can be found in the literature, for instance EnvZ in *E. coli* osmolarity regulation [28]. Notice also that the self-mediated phosphorylation can be found in the epidermal growth factor receptor (EGFR) [29, 30]. While the possibility of the practical implementation of the basic ACR controller remains open, we focus on theoretic aspects of the controller in this manuscript.

Assuming that the dynamics associated with the ERK model and the basic ACR system in Figure 2a follows mass-action kinetics, we use $\mu = 2$ and $\theta = 1$. This implies that for any input $E_{\text{tot}}, F_{\text{tot}}$ and S_{tot}, the steady state of F is 2. The convergence of F to 2 is also theoretically proven, see Section 8.1 in the Supplementary material. Thus in the controlled system, the concentration of F converges to 2, unlike the uncontrolled original ERK model which has different steady state concentrations of F for different values of F_{tot}, as described in Figure 2b (left) and 2c (left). As a result, S_{11} in the controlled system has identical dose response regardless of the value of F_{tot} (right plot in Figure 2c). On the other hand, the S_{11} dose responses are different in the original system (the right plot in Figure 2c).

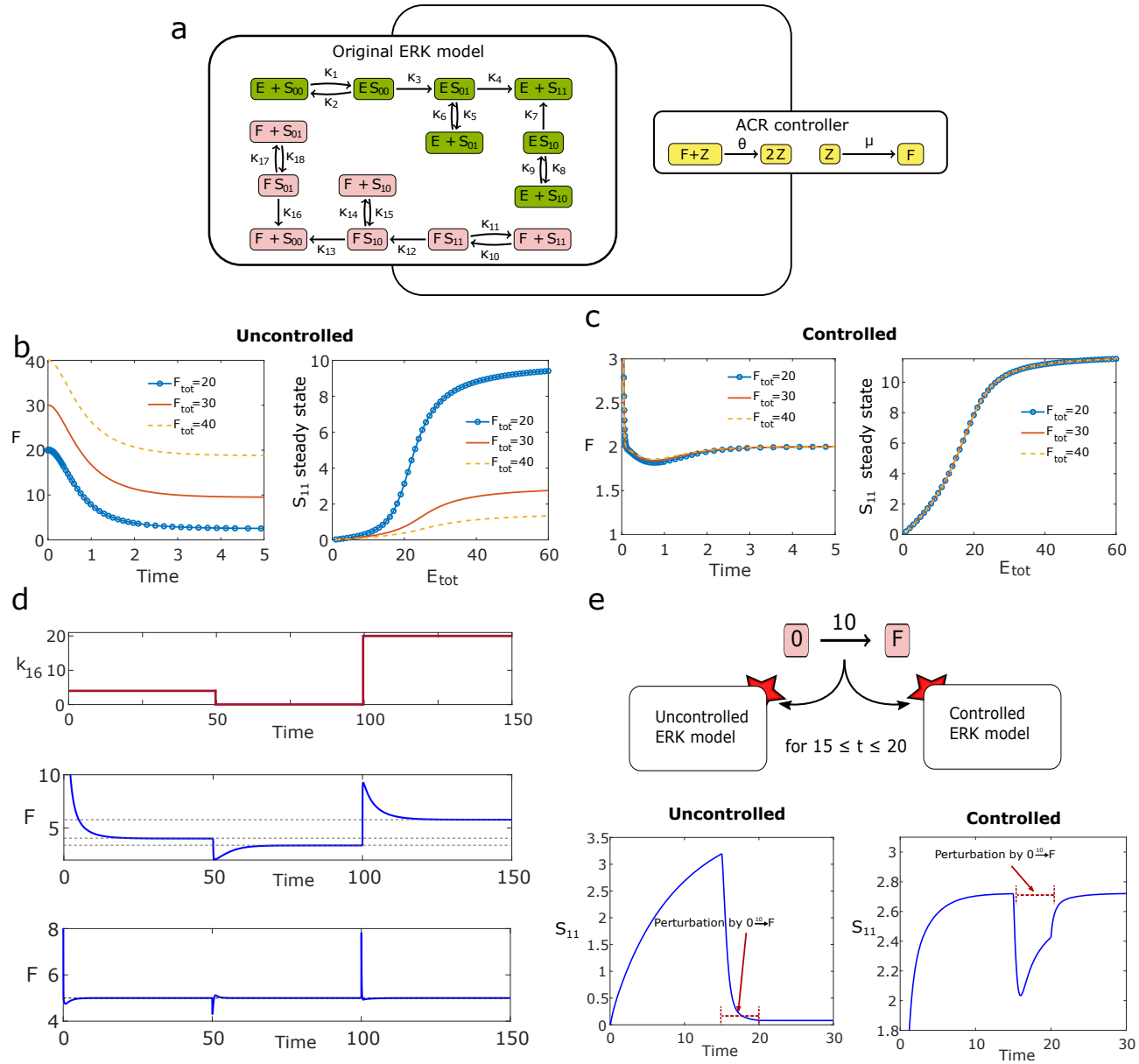

Figure 2: **a.** Reaction network of an ERK system originally proposed in [25], together with the basic ACR controller. Parameters used are $k_1 = 3, k_2 = 2, k_3 = 1, k_4 = 2, k_5 = 1, k_6 = 3, k_7 = 2, k_8 = 5, k_9 = 3, k_{10} = 2, k_{11} = 2, k_{12} = 3, k_{13} = 1, k_{14} = 3, k_{15} = 1, k_{16} = 1, k_{17} = 4, k_{18} = 4$ for the original ERK system, and $\theta = 1$, $\mu = 2$ for the ACR controller. **b.** (Left) Time evolution of F in the original system without the ACR controller. (Right) Dose response of active ERK S_{11} as a function of total kinase E_{tot} for the original system. Initial conditions are $S_{00} = 50$, $E = E_{tot}$, $F = F_{tot}$, and zero for all other species. **c** (Left) Time evolution of F in the controlled system. (Right) Dose response of S_{11} as a function of E_{tot} for the controlled system. Initial conditions are $S_{00} = 50$, $Z = 50$, $E = E_{tot}$, $F = F_{tot}$, and zero for all other species. **d.** Robust perfect adaptation of F in the controlled system. We set the initial conditions as $S_{00} = 30, E_{\text{tot}} = 30, F_{\text{tot}} = 30, z(0) = 30$ and zero for all other species. **e.** The dynamics of S_{11} in the original (left) or controlled (right) system which is transiently perturbed by reaction $0 \xrightarrow{10} F$ for $t \in [15, 20]$.

2.2 Robust Perfect Adaptation

One of the main purposes of the control with an ACR system is to create *robust perfect adaptation* for the target species. A system possesses perfect adaptation if the output of the system returns to the pre-stimulus level when persistent changes in the input present. Furthermore, if the system achieves perfect adaptation independently of the system parameters, it is said to have robust perfect adaptation (RPA) [31–34].

Here we show that species F in the controlled ERK model in Figure 2 admits RPA. We persistently disturb the parameters by changing them in time such as k_{16} as shown in Figure 2d (top). As expected, the concentration of phosphatase F converges to different values for each perturbation as depicted in Figure 2d (middle). However as Figure 2d (bottom) shows, the controlled ERK system has robustness to the perturbations for F as its concentration converges to the set-point $\frac{\mu}{\theta} = 2$ regardless of parameter values. This RPA for F basically arises because the steady state of F is completely determined by the two reactions in the ACR controller, and it is independent on the parameters k_i of the original ERK system. More details are in Section 6 of the supplementary material.

In addition to RPA, we also show that the controlled system is robust to a transient change of the system structure. We perturb the system by turning on an additional reaction $0 \xrightarrow{10} F$ for time $[15, 20]$. The S_{11} concentration in the uncontrolled system initially converges, but it immediately responds to the transient in-flow as F is produced during that time interval (Figure 2e (left)). The concentration does not return to the previous steady state value after the transient perturbation is turned off. In the controlled system, the phosphorlyated protein S_{11} also responds when the transient in-flow is switched on at $t = 15$ as show in Figure 2e (right). However, it is quickly driven back to the steady state after the perturbation is switched off at $t = 20$. This robustness to the transient structural disturbance stems from the fact that the controlled system admits a single positive steady state concentration for the target species F regardless of the input states. Hence when the transient inflow is turned off, F in the controlled system converges to the set-point wherever the current state of the system has been driven by the perturbation.

2.3 Control of Additional Species

Recall that the basic ACR system (1) consists of the control species Z and the target species A. The fact that Z only directly controls A may impose limitations in some situations. We show in the following example that an ACR controller with reactions involving other network species can provide better performance.

For example, consider the following reaction network where no conservation relations exist:

$$A + B \xrightarrow{\kappa_1} \emptyset \begin{array}{l} \xrightarrow{\kappa_2} A \\ \xrightarrow{\kappa_3} B \end{array} \tag{7}$$

In this system, two proteins A and B are constantly produced but also degrade each other. Using the parameters $\kappa_1 = 1, \kappa_2 = 3, \kappa_3 = 5$, the concentration of A decays toward zero as shown in Figure 3d. Despite the addition of the basic ACR system, the concentration of A still decays to

zero as shown in Figure (3)e. See Section 8.2 in the Supplementary Material for additional details about this system, including the existence of positive steady states.

We design the expanded controller shown in Figure 3c to include both A and B in the reactions. It can be verified that the mass-action system associated with this controller is ACR, with ACR species A. This is because the additional reactions $Z + B \rightleftharpoons Z$ do not change Z, so that the equation for Z is the same as in the base ACR model. Such reactions that have no contribution to the control species are also used for the antithetic integral controller in [7, 8]. Using $\theta = 1, \mu = 5, \alpha_1 = 2, \alpha_2 = 1$, one can see that this controller steers the positive steady state concentration of A to 5 for different initial conditions (Figure 3f). See Section 7 in the Supplementary Material for control of general 2-dimensional systems.

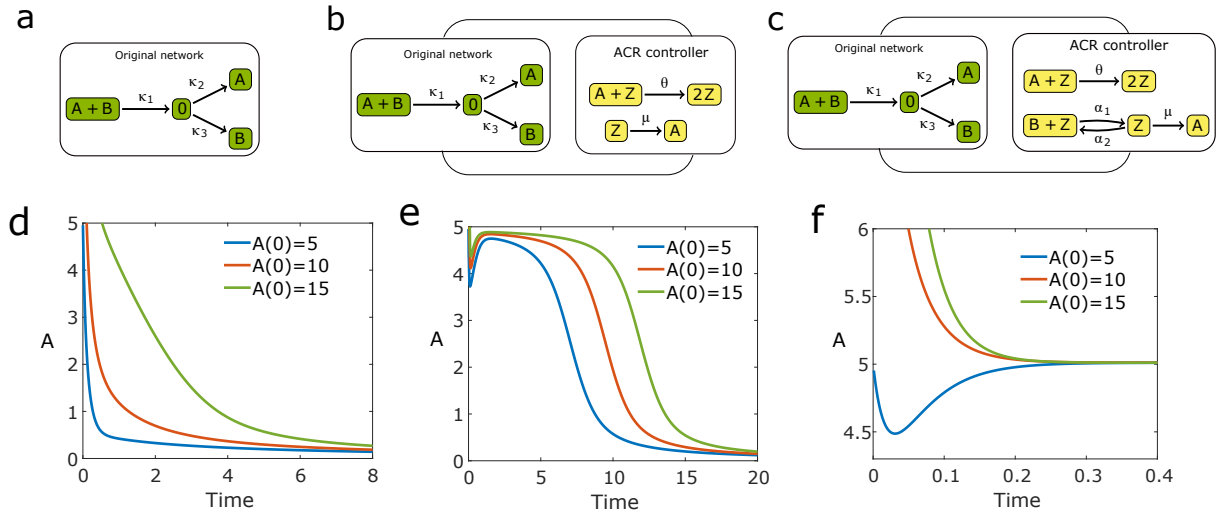

Figure 3: **a.** Original network, using parameters $\kappa_1 = 1, \kappa_2 = 3$ and $\kappa_3 = 5$. **b**. The basic ACR system is added to the original system, with $\mu = 5, \theta = 1$. **c.** Expanded ACR controller, with $\alpha_1 = 2$ and $\alpha_1 = 1$. **d.** The concentration of A converges to zero in the original system. **e.** The basic ACR system in 3b fails to control A, as A still converges to zero for different initial values. **f.** The concentration of A is driven to the set-point 5 with each initial condition, for the controlled system in c.

2.4 Stochastic Control Using an ACR Module

When a system contains species with low copy numbers, the intrinsic noise considerably affects the system dynamics. Therefore we model the system stochastically using a Markov process. This continuously evolving Markov process defined on a multi-dimensional integer grid has state-dependent transition rates (for more detail see Section 4.1 in the Supplementary material). In the context of stochastic control, recent work by Mustafa Khammash and others has proposed controlling a target species by adding four reactions. While that framework allows to control the mean of the target species, there could be significant variability in its noise. Our ACR approach makes use of topological properties of the original network to approximate the full distribution of the target species.

In stochastic networks, if one of the species reaches zero copies, then a subset of the reactions in the system would be turned off, potentially preventing the species from ever being produced. Such an extinction event can take place for Z in the basic ARC controller as well as many other

ACR systems [21]. In order to avoid this situation, we design the basic ACR system with sufficiently high copies of the control species. More generally, we assume that all species are classified into two types: highly abundant species such as control species Z which are of order N for a scaling parameter N, and low abundance species of constant order. We also scale the parameters of the controlled system to make all the reaction propensities of constant order. Under this same scaling, Enciso [19] used the technique of species 'freezing' for an ACR system to generate a reduced network of low abundant species. It was further shown that if the reduced network has zero deficiency and is weakly reversible, then an ACR species of low order tends to follow a Poisson distribution centered at its ACR value, as time t and the scaling parameter N go to infinity.

The work in [19] approximated the distribution of the target species with the help of a reduced stochastic model, which is the limit of the original stochastic network using a multiscaling procedure. Similar types of approaches have been studied using different system scaling, network topological conditions or state space truncations [2, 3, 20, 35–38]. The multiscaling assumption in [19] is somewhat special in that all reaction propensities have constant order of magnitude up to finite time.

Given a stochastic chemical reaction network, we now add an ACR controller and use the scaling procedure described above in order to study the resulting controlled system. To exemplify this we consider a model describing the dynamics of a receptor binding to a ligand and generating a downstream response (Figure 4a). Many important biology models involve receptor-ligand interactions such as signal transduction, physiological regulation, and gene transcription. In this case a ligand L binds to an inactive receptor R_0 on the cell membrane, converting it into an active receptor R. Two active receptors are dimerized, forming the species D which is phosphorylated sequentially in three different locations. The triphosphorylated dimer D_3 transmits the signal inside the cell by activating another protein P as shown in Figure 4d. We control the inactive receptor R_0 using the basic ACR system, in order to control the desired amount of active protein P^*.

Once again, the practical implementation of such a system must depend on the specific receptor. We suggest a possible implementation as follows: suppose that a second ligand, called an antagonist, binds to the receptor forming a molecule Z, which prevents the binding of the original ligand (see Figure 4d). Suppose the complex Z facilitates the recruitment of another antagonist to produce another copy of Z, leading to the reaction $Z + R_0 \to 2Z$. The reaction $Z \to R_0$ simply represents the natural unbinding of the antagonist from R_0. Another option could be to think of Z as a misfolded form of R_0, and of the reaction $Z + R_0 \to 2Z$ as a prion-like effect where a misfolded receptor makes it more likely that a second receptor will misfold. In any of these cases, the introduction of an new molecule into the system (the antagonist or the misfolded protein) leads to two additional reactions that control the network.

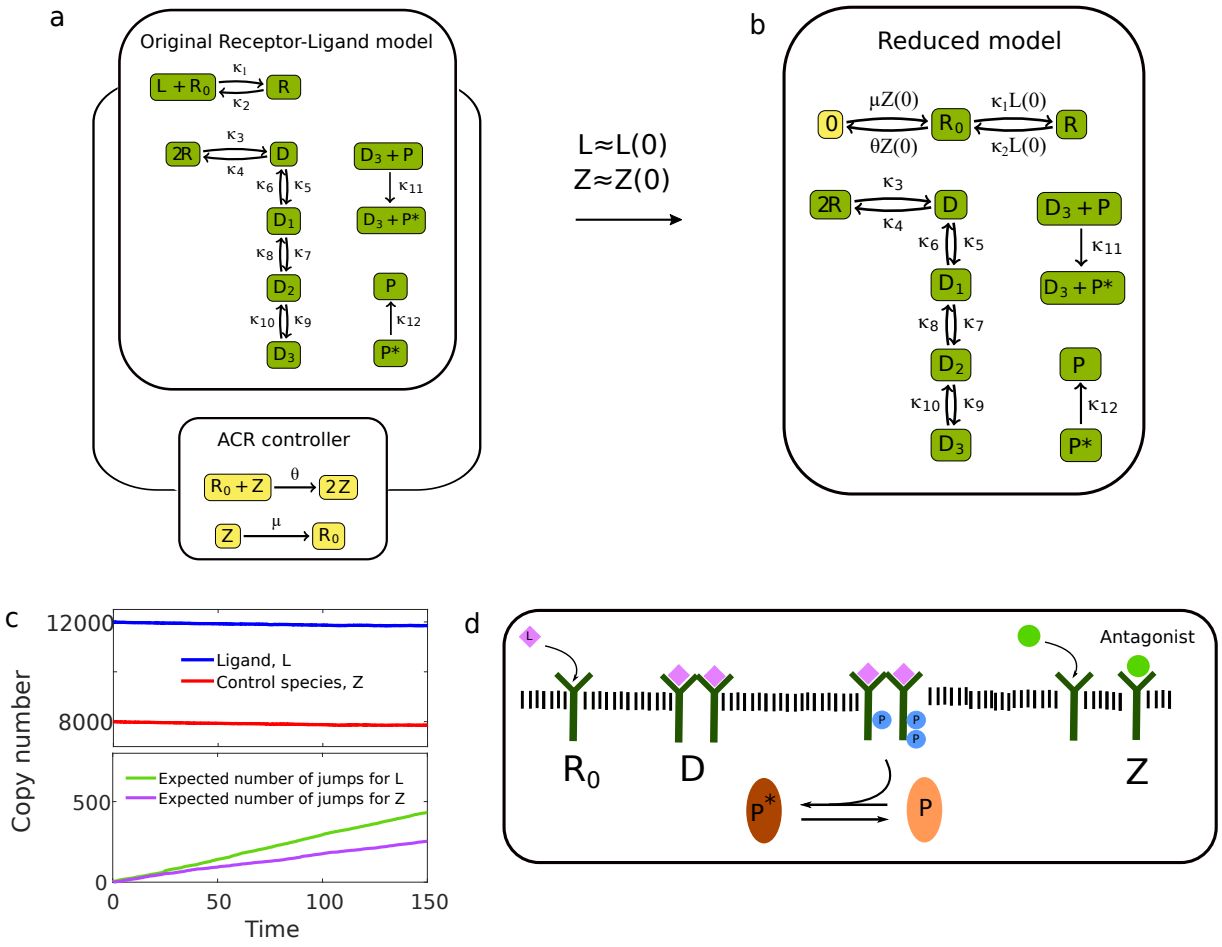

Figure 4: **a.** Reaction network for the receptor-ligand pathway (green) and the ACR controller (yellow). **b.** Reduced model obtained by freezing L and Z at their initial values, respectively. **c.** Stochastic time evolution of the copy numbers of L and Z, highlighting the small net change of L and Z in the system by time $t = 150$. **d.** A schematic picture for the receptor-ligand model and the ACR controller

We let the system start with initial counts $L(0) = 1500, Z(0) = 1000, R_0(0) \leq 50$ and the initial copy numbers of all the other species equal to zero. Hence species L and Z are the high abundance species of order $N = 1000$ and the other species are of low abundance.

As mentioned above, the main idea of the control for this system is to approximate the distribution of R_0 by the reduced network in Figure 4b, which we now explain. Parameters are chosen as $\kappa_1 = 0.82 \times 10^{-3}, \kappa_2 = 1.37, \kappa_3 = 1.41, \kappa_4 = 1.79, \kappa_5 = 1.02, \kappa_6 = 1.36, \kappa_7 = 1.97, \kappa_8 = 1.11, \kappa_9 = 1.55, \kappa_{10} = 1.01, \kappa_{11} = 1.34, \kappa_{12} = 0.5, \theta = 10^{-3}$ and $\mu = 5 \times 10^{-3}$. In order to arrive to this parameter set, parameters κ_2 through κ_{12} were randomly chosen in the range $[1, 2]$. Parameters κ_1, θ and μ are associated with reactions involving high abundance species L and Z, and they were chosen of order $\frac{1}{N}$ so that the reactions $L + R_0 \to R, Z + R_0 \to 2Z$ and $Z \to R_0$ have constant order propensities under mass-action kinetics. Details of the mass-action propensity computations are provided in Section 10.1 in the Supplementary Material. Because of the low propensities of the reactions relative to N, the expected change of species L and Z by $t = 150$ are much smaller than the copy numbers of L and Z as Figure 4c shows. By neglecting the relatively small number of fluctuations for L and Z shown in Figure 4c, we can freeze them at their initial

counts and obtain a reduced system in Figure 4b.

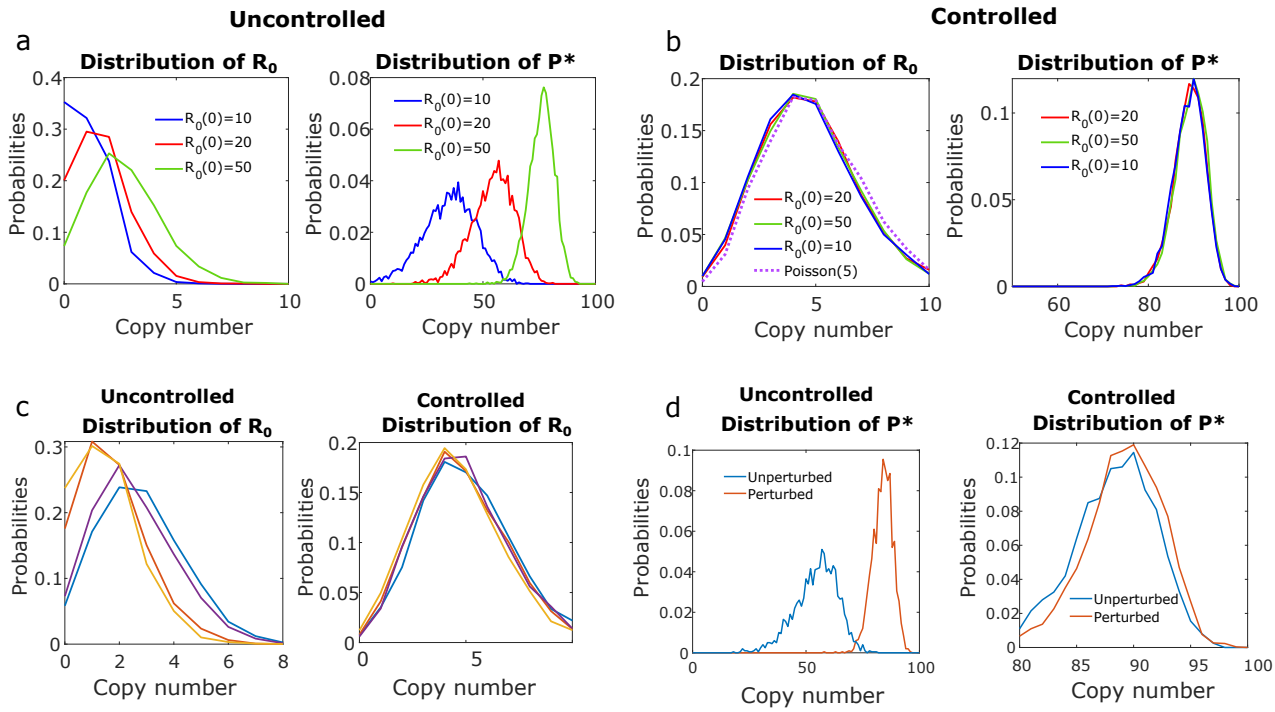

Figure 5: Gillespie simulations [39] for the distribution of R_0 and activated protein P^*. We use the initial values $L(0) = 1500, P(0) = 100, R(0) \leq 50$, and the remaining species have zero initial values. For the ACR controller, we set $Z(0) = 1000$. **a.** For the uncontrolled system, distribution at time $t = 150$ of inactive receptor R_0 (left) and active protein P^* (right). **b.** (Left) For the controlled system, distribution at time $t = 150$ of R_0 (left) and P^* (right). **c** and **d.** Robustness of the system to randomized parameter perturbations and a transient reaction perturbation, setting $R_0(0) = 20$. **c.** Four distributions of R_0 obtained by simulations of the uncontrolled (left) and controlled (right) receptor-ligand model with randomly perturbed system parameters. **d.** Unperturbed and perturbed distributions of P^* by transiently switched-on reaction $0 \xrightarrow{2} R_0$ for $t \in [50, 80]$ in the uncontrolled (left) and controlled (right) receptor-ligand model.

Recalling that R_0 in the receptor-ligand network in Figure 4a and its associated reduced network 4b behave similarly over time, we carry out an analysis of the reduced network. We initially ignore the reactions $D_3 + P \to D_3 + P^*$ and $P^* \to P$ because they only affect the proteins P, P^* without an effect on other species. The resulting network is reversible, and it has zero deficiency since

$$n - \ell - s = 8 - 2 - 6 = 0,$$

where n is the number of complexes, ℓ is the number of connected components, and s is the rank of the stoichiometry matrix. The distribution of R_0 in the reduced network converges to a Poisson distribution in the long run [1]. Depending on the number of states of the stochastic system, the long-term distribution could be a truncated Poisson distribution. However, the reaction in the ACR controller are always reduced to the inflow and the outflow of the target species in the reduced system. Hence the long-term distribution is always approximated a Poisson distribution. See Section 9.3 in the Supplementary Material for a more rigorous statement.

Furthermore, the rate of the Poisson distribution associated with R_0 is determined by the steady state value of the corresponding deterministic system [1]. Since the rate is equal to the mean of the Poisson distribution, the mean of R_0 is close to $\frac{\mu}{\theta}$ in the long run. Thus species R_0 in the controlled system shown in Figure 4a at a sufficiently large finite time t is well approximated by the Poisson distribution centered at $\frac{\mu}{\theta}$. This is shown in Figure 5b (left) at $t = 150$, where for any input R_0 the distribution seems almost Poisson($\frac{\mu}{\theta}$). Consequently the protein P distribution is also robustly stabilized as shown in Figure 5 b (right). On the other hand both mean and variance of R_0 in the original system vary with respect to different inputs (Figure 5a, left), and this causes the distribution of P^* to change accordingly (Figure 5a, right).

In an additional analysis, we study the convergence speed of the distribution of the reduced system towards a stationary distribution in Section 10.1 of the Supplementary Material. The underlying mathematical framework, with an emphasis on the accuracy of the approximation between the controlled network and the reduced system, is further described in our follow-up paper [40].

For the receptor-ligand system, the basic ACR module also robustly controls the target species to perturbations. We perturb the parameters κ_i in Figure 5 using the equation $\kappa_i' = \kappa_i + r_i$, where the r_i are sampled from a uniform distribution on the interval $[0, 3]$. As shown in Figure 5c (left), the uncontrolled system generates distinct distributions of R_0 at $t = 150$ for randomly perturbed parameters in each simulation. On the other hand, the distributions of R_0 at $t = 150$ generated by the controlled system with the same parameters closely approximate the Poisson distribution with mean $\frac{\mu}{\theta} = 5$, as shown in Figure 5c (right).

Plots in Figure 5d show how P^* robustly behaves with a transient perturbation in the controlled system. We perturb the system with a reaction $0 \xrightarrow{2} R_0$ only for time $t \in [50, 80]$. Because of this additional input, the distribution of P^* at $t = 150$ is shifted to the right for the uncontrolled system (Figure 5d, left). However for the controlled system, Figure 5d (right) shows that its distribution is robust to the transient perturbation.

2.5 Stochastic Control Using a Hybrid Approximation

Recall that in the receptor-ligand system in Section 2.4, the fluctuation of species L and Z in the concentrations are negligible since the reaction propensities are small compared with their concentration. However, many classical studies of stochastic systems eliminate this assumption of small reaction propensities, see for instance the classical work by Kurtz [22]. Reaction propensities could also have different orders of magnitude with respect to N. In such cases, the stochastic system is modeled under a multiscaling regime, and its behaviour can be studied using a hybrid deterministic-stochastic system [20, 23, 24, 41]. In a hybrid system, the counts of some species change stochastically while the concentrations of the other species change continuously. We modify the basic controller in order to control such a hybrid system. In this section, using the finite time stationary distribution approximation in [20], we show that an expanded basic ACR system can be used to control a stochastic system under more general scaling.

As an example, we provide a dimer-catalyzer model in Figure 6a. In this system the initial copy number of species X^*, X_1, C, C_p and C_{pp} are all of order $N = 1000$. Hence using mass action kinetics all the reactions have order N propensities. Using the framework established by Anderson et al [20], we approximate the original model with the hybrid system in Figure 6d. The stochastic part of the hybrid system has zero deficiency and is weakly reversible so that the distribution of the

target species X is Poisson at a finite time $t = 5$ [1, 20]. The stochastic and deterministic parts are coupled, as the mean $m(t)$ of X at finite time t is determined by the dynamics of the deterministic system as depicted in Figure 6e. A flux balance analysis implies that $m(t) = \dfrac{k_1 x*(t) + \mu z(t)}{k_2 c_{pp}(t) + \theta z(t)}$. In Section 10.2 of the Supplementary Material, we show that $m(t)$ converges to the desired value $\dfrac{\mu}{\theta}$, as $t \to \infty$, when the initial concentration of Z is sufficient. The mean $m(t)$ actually converges to $\dfrac{\mu}{\theta}$ quickly as shown in Figure 6e. Therefore unlike the distribution of X in the original system as shown in Figure 6b, the distribution of X in the controlled system is approximately Poisson centered at $\dfrac{\mu}{\theta} = 5$ at time $t = 5$ for randomly sampled parameters κ_i (6c).

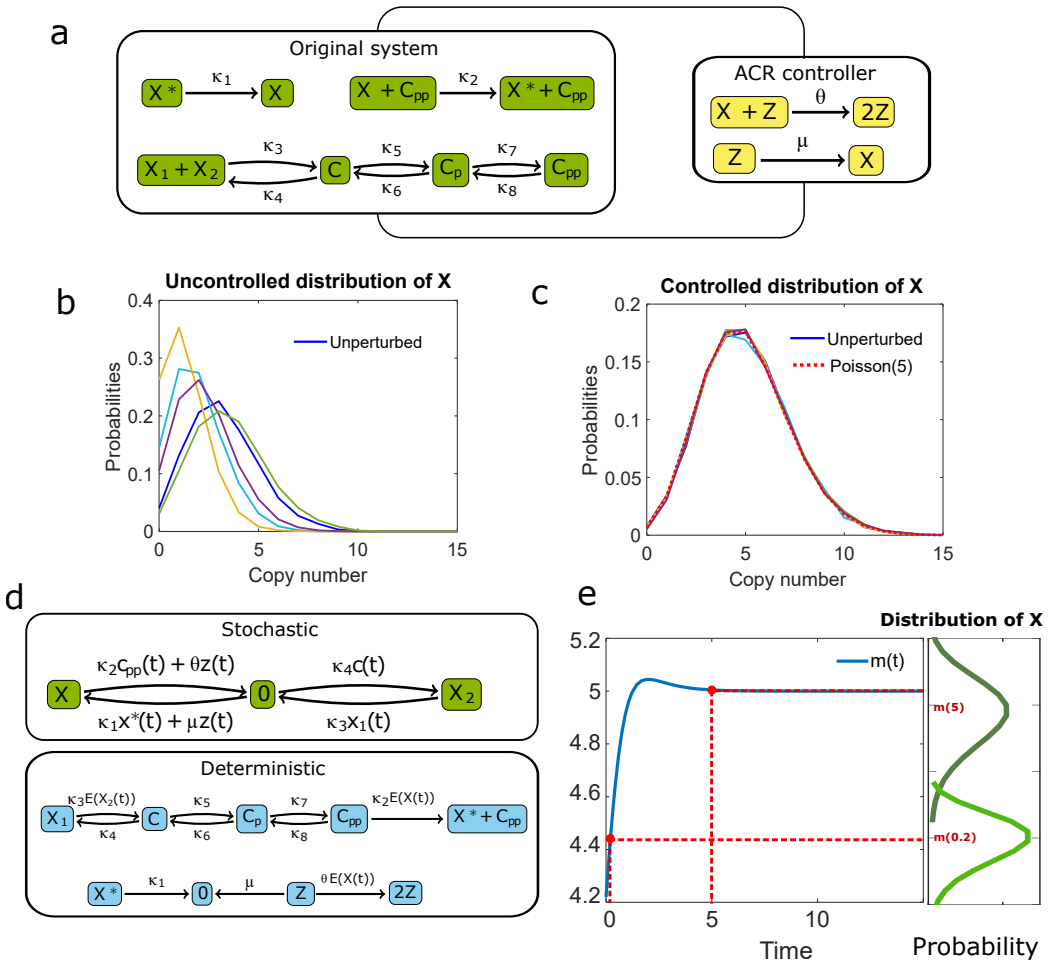

Figure 6: Dimer-catalyzer model with high reaction rates of order N **a.** The original model and the ACR controller. Parameters are $\kappa_1 = 1.38, \kappa_2 = 1.58, \kappa_3 = 1.19, \kappa_4 = 1.01, \kappa_5 = 1.17, \kappa_6 = 1.92, \kappa_7 = 1.11$ and $\kappa_8 = 1.88$. The parameters are sampled uniformly randomly in $[1, 2]$. We use $\mu = 5$ and $\theta = 1$ for the ACR controller. **b.** Distribution of X at $t = 5$ in the uncontrolled system in Figure 6a with both the chosen parameters and randomly perturbed parameters. **c.** Distribution of X at $t = 5$ in the controlled system using both the chosen parameters and randomly perturbed parameters. Red dotted line indicates the Poisson distribution with the rate 5. **d.** The controlled system is approximated with a hybrid model consisting of stochastic and deterministic parts. **e.** The mean $m(t)$ of the distribution of X is displayed (blue solid line) along with the distribution of X at times $t = 0.2$ and $t = 5$.

3 Discussion

Absolutely robust networks have the property that the steady state value of a target species is independent of the total mass of the system. In this paper we have provided a class of controllers based on absolutely robust networks. We define a control species that interacts with the target species, embedding an absolutely robust network into the given network to enforce target species robustness. For deterministically modeled networks, this type of controller not only stabilizes the target species at the desired value by tuning the parameters of the ACR controller, but also makes the species robustly adapted to parameter perturbations and a transiently supplied additional reactions. We demonstrate control for deterministic system through an ACR system with an ERK model. We illustrate some of our results with the so-called base ARC controller, but we show that other ACR networks can be also be used.

We also show that ACR controllers have the ability to control stochastic networks. The need for control stochastic system is becoming clear in many disciplines of systems and synthetic biology, particularly given the low species counts present in many individual cells. The average of a species concentration is a deterministic quantity of a stochastic system, thus one might think that a controller used for a typical deterministic system could also implement stochastic control. However for a nonlinear system, studying the dynamics associated with the averages requires nontrivial tools such as moment closure [42]. Even if mean control is valid with a given controller, the system may be still out of control if noise is not properly accounted for. Furthermore, because the associated stochastic system describes molecular counts of each species instead of concentrations, some species might reach a zero state and lead to an extinction event.

As a result, for the control of stochastic systems it can be helpful to use advanced mathematical tools such as theoretical analysis of chemical reaction networks. Using an ACR controller for stochastic systems here involves two main mathematical tools, multiscaling model reduction and deficiency zero theorems. To avoid a potential breakdown of a controller because of lack of reactants, we design an ACR controller with high copies of the control species. Using the tools above, we show that a species of interest in the controlled system is roughly Poissonian with tunable mean and variance. Combining the multiscaling model reduction and the zero deficiency condition, we show that a simple ACR system can control both mean and variance of an inactive receptor in stochastic receptor-ligand system as the distribution of the inactive receptor roughly follows a Poisson distribution centered at the desired value. The controlled stochastic system also admits robust perfect adaptation as does the corresponding deterministic system.

We note that the basic ACR controller used throughout this paper has a connection to classical control theory, as it admits a non-linear integral feedback that is a well-studied characteristic of robustly adapted systems [31, 32, 34]. Integral feedback loops arise in many important biological phenomena such as bacterial chemotaxis, photoreceptor responses, or MAP kinase activities. For the simple mass-action ACR system

$$Z + X \xrightarrow{\theta} 2Z, \quad Z \xrightarrow{\mu} X, \tag{8}$$

the concentration of Z satisfies $\frac{d}{dt}z(t) = z(t)(-\mu + \theta x(t))$. Dividing by $z(t)$ and integrating on both sides, we obtain

$$\log z(t) = \log z(0) + \int_0^t (\theta x(s) - \mu)ds, \tag{9}$$

which is a non-linear integral feedback relation. Such types of integral feedback loops appear in many different biochemical systems [7, 9, 33, 43, 44].

One of the major issues on synthetic controllers is the practical implementation of the proposed controller. Aoki et al. [8] show that an antithetic controller could be constructed using two control proteins, σ factor SigW and anti-σ factor RsiW, in an emphE. coli plasmid implementation. For an ACR controller, it remains an open question whether its design is practically feasible *in vivo* or *in vitro*. One key for synthesizing it is the bifunctionality of an enzyme that potentially brings ACR to the system, as it has been observed for other ACR applications [45, 46]. Notice that the control species Z mediates both production and degradation of the target species X in the basic ACR controller. We have suggested some ideas for implementing ACR controllers in our examples. The control species could be obtained by phosphorlyation of a bifunctional target species, by antagonist ligand binding, or by a form of protein misfolding.

As sufficient network architectural conditions for ACR property have been shown for example in the regulation of osmolarity in bacteria [5], designing more general ACR controllers could be feasible. Therefore we believe that this new approach introduced in this paper could help control other biochemical networks in a way that takes into account stochastic effects.

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Supplementary Material

4 Chemical reaction network theory

4.1 Reaction networks

In this section, we provide mathematical models associated with biochemical systems that we use in the main manuscript, starting with the introduction of reaction networks. A biochemical system can be described with a reaction network, which consists of constituent species, complexes that are combinations of species, and reactions between complexes. A triple $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ represents a reaction network where $\mathcal{S}, \mathcal{C}$ and $\mathcal{R}$ are collections of species, complexes and reactions, respectively.

Example 4.1. *Consider the following reaction network describing a substrate-enzyme system.*

$$S + E \rightleftharpoons SE \to E + P,$$

For this reaction network, $\mathcal{S} = \{S, E, SE, P\}$, $\mathcal{C} = \{S + E, SE, E + P\}$ *and* $\mathcal{R} = \{S + E \to SE, SE \to S + E, SE \to E + P\}$. △

Regarding a reaction network as a directed graph, each connected component is termed a *linkage class*. A subset Q of complexes in a linkage class is a *strongly connected component* if and only if for any two complexes $y, y' \in Q$, there exists a path of directed edges connecting from y to y'. If every linkage class in a network consists of a single strongly connected component, then the network is *weakly reversible*. By the definition, in a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$, the set of complexes $\mathcal{C}$ can be decomposed into disjoint linkage classes. Allowing that a single complex can be a strongly connected component, every linkage class is decomposed into disjoint strongly connected components.

For example, for the following network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$

$$\emptyset \rightleftharpoons C, \quad A \rightleftharpoons B \to A + B \to 2C \rightleftharpoons B + C, \tag{10}$$

there are two linkage classes $\{\emptyset, C\}$ and $\{A, B, A+B, 2C, B+C\}$. Linkage class $\{\emptyset, C\}$ consists of a single strongly connected component. Linkage class $\{A, B, A + B, 2C, B + C\}$ has three strongly connected components $\{A, B\}, \{A + B\}$ and $\{2C, B + C\}$.

Each strongly connected component is further classified into two categories. For a strongly connected component Q, if there is no path of directed edges connecting from $y \in Q$ to $y' \notin Q$, then Q is a *terminal connected component*. Otherwise, Q is a *non-terminal connected component*. A complex contained in a terminal connected component is called a *terminal complex*, otherwise it is called a *non-terminal complex*. In (10), strongly connected components $\{\emptyset, C\}$ and $\{2C, B+C\}$ are terminal connected components, and the others are non-terminal connected components.

We introduce a domain on which the dynamical system associated with a reaction network is defined.

Definition 4.1. *Let* $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ *be a reaction network. For a* $x_0 \in \mathbb{R}^d_{>0}$*, we call a set* $S_{x_0} = x_0 + span\{y' - y : y \to y' \in \mathcal{R}\} \cap \mathbb{R}^d_{>0}$ *the stoichiometry class.*

4.2 Dynamical systems

For a dynamical system of a reaction network, a reaction rate constant κ for each reaction $y \to y'$ gives a weight on each reaction, and we denote $y \xrightarrow{\kappa} y'$ to incorporate the rate constant. With a collection of rate constants $\mathcal{K}$, we denote the associated dynamical system for $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ by $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$.

For mathematical models of reaction networks, we typically assume that the associated system is spatially well-stirred. In this case the usual mathematical model for a reaction network is either a system of ordinary differential equation or a continuous-time, discrete-space Markov process. When each species has high copy number so that intrinsic noise can be averaged out, the concentration vector $x(t)$ of species in a reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ is typically modeled with a deterministic network system

$$\frac{d}{dt}x(t) = \sum_{y \to y'} \kappa_{y \to y'} \eta_y(x(t))(y' - y), \tag{11}$$

where $\eta_y : \mathbb{R}^d_{>0} \to \mathbb{R}_{>0}$ is a rate function associated to a reaction $y \xrightarrow{\kappa_{y \to y'}} y'$. One of the prevalent choice of the rate function is *mass action kinetics* which defines $\eta_y(x) = x^y$, where $u^v = \prod_{i=1}^d u_i^{v_i}$ for two vectors $u, v \in \mathbb{R}^d_{\geq 0}$.

The intrinsic stochasticity of a system is considered when each species in a reaction network system has low copy number. For the usual stochastic model, we use a continuous time, discrete state space Markov process $X(t) \in \mathbb{Z}^d_{\geq 0}$ defined on $\mathbb{Z}^d_{\geq 0} = \{z \in \mathbb{Z}^d : z_i \geq 0 \text{ for each } i\}$. The transitions of X are determined by the reaction vectors. Letting $h(t)$ be a function such that $\lim_{t \to 0} \frac{h(t)}{t} = 0$, the transition probabilities are defined as

$$P(X(t + \Delta t) = z + y' - y \mid X(t) = z) = \sum_{\substack{\bar{y} \to \bar{y}' \\ \bar{y}' - \bar{y} = y' - y}} \kappa_{\bar{y} \to \bar{y}'} \lambda_{\bar{y} \to \bar{y}'}(z)\Delta t + h(\Delta t), \tag{12}$$

where $y' - y$ is a reaction vector associated with a reaction $y \to y'$, and $\lambda_{y \to y'} : \mathbb{Z}^d_{\geq 0} \to \mathbb{R}_{\geq 0}$ is the *reaction intensity* representing how likely the associated reaction $y \to y'$ fires.

The usual choice of the propensity functions for a stochastic network system $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ is

$$\lambda_{y \to y'}(x) = x^{(y)}, \tag{13}$$

where $u^{(v)} = \prod_{i=1}^d \frac{u_i!}{(u_i - v_i)!} \mathbf{1}_{\{u_i \geq v_i\}}$ for $u, v \in \mathbb{Z}^d_{\geq 0}$. This choice of the propensity function is *stochastic mass-action kinetics*. In both this supplementary material and the main text, we model both the deterministic and the stochastic dynamical system under mass action kinetics. Letting $\mathcal{K}$ be the set of reaction intensities associated with $\mathcal{R}$, the quadruple $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ defines a (either deterministic or stochastic) dynamical system associated with the reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$.

An infinitesimal behavior of the associated X can be described with the infinitesimal generator $\mathcal{A}$ [47],

$$\mathcal{A}V(x) = \lim_{h \to 0} \frac{E_x(V(X(h))) - V(x)}{h} = \sum_{y \to y' \in \mathcal{R}} \lambda_{y \to y'}(x)(V(x + y' - y) - V(x)), \tag{14}$$

for a function $V : \mathbb{Z}^d_{\geq 0} \to \mathbb{R}$, where E_x denotes the expectation of the process whose initial point is x.

4.3 Deficiency zero theory

The deficiency of a reaction network is a positive integer determined solely by the structure of the network regardless of parameter values. Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a reaction network with m complexes and ℓ linkage classes. Let further s be the rank of the stoichiometric matrix whose i-th column is given by i-th reaction in $\mathcal{R}$. The deficiency δ is equal to

$$m - \ell - s.$$

There are a couple of interpretations of the deficiency. First, we can represent the deterministic system (11) as

$$\frac{d}{dt}x(t) = Y A_{\mathcal{K}} \psi(x(t))$$

with a stoichiometry coefficient matrix Y, rate constant matrix $A_{\mathcal{K}}$ and the rate function $\phi(x)$ (See [6] for more details). Then the deficiency δ of a network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ satisfies

$$\delta = dim(Ker(Y) \cap Im(A_{\mathcal{K}})).$$

Second, the deficiency roughly stands for redundancy of the network in the following sense. Consider the following two networks,

$$\emptyset \rightleftharpoons A \quad \text{and} \quad \emptyset \rightleftharpoons A \rightleftharpoons 2A.$$

The deficiency of the left reaction network is $0 = 2 - 1 - 1$. The deficiency of the right reaction network is $1 = 3 - 1 - 1$. This difference stems from the additional reaction $A \rightleftharpoons 2A$ in the right network. The gain and loss of one A species is already realized with reaction $\emptyset \rightleftharpoons A$. Hence reaction $A \rightleftharpoons 2A$ is redundant.

Zero deficiency combined with weak reversibility of reaction networks implies very strong characteristics of the associated system dynamics for both deterministic models and stochastic models.

Theorem 4.1 (Horn 1972 [16], Feinberg 1972 [17]). *Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a weakly reversible reaction network with zero deficiency. Then for any choice of rate parameters, the associated deterministic dynamics endowed with the mass-action kinetics admits a unique locally asymptotic stable positive steady state at each stoichiometry class.*

The stationary distribution of the associated stochastic process is fully characterized for a weakly reversible network which has zero deficiency.

Theorem 4.2 (Anderson, Craciun and Kurtz 2010 [1]). *Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a weakly reversible reaction network with zero deficiency. Then for any choice of rate parameters, the associated Markov process endowed with the stochastic mass-action kinetics admits a stationary distribution, and it is*

a product form of Poissons (or constrained Poissons). That is, for each $x \in Z^d_{\geq 0}$ in the state space, the stationary distribution π satisfies

$$\pi(x) = M \prod_{i=1}^{d} \frac{c_i^{x_i}}{x_i!}$$

where $c = (c_1, c_2, \ldots, c_d)$ is a steady state of the deterministic counterpart and M is the normalizing constant.

For control of a stochastic model, we use Theorem 4.2 to find an approximation of a target species in a controlled system. Details about this procedure is state in Section 9.

5 ACR systems and ACR controllers

In this section we introduce the absolute concentration robustness (ACR) of a reaction network. In order to make use of ACR systems to design a controller, we consider a special class of ACR networks, and then we introduce a precise definition of an ACR controller.

Definition 5.1. *Let $\hat{x}$ be a solution to the deterministic network system $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ such that*

$$\frac{d}{dt}\hat{x}(t) = \hat{f}(\hat{x}(t)).$$

Suppose this system admits a positive steady state. If there exists a species $X_1 \in \hat{\mathcal{S}}$ such that the values of X_1 at any positive steady states are all identical, then $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ is called an ACR network system. Furthermore, the species X_1 and the identical positive steady state value of X_1 are called an ACR species and an ACR value, respectively. Especially if the deterministic model is equipped with mass-action kinetics, the system is called a mass-action ACR network system.

In some special cases, the ACR property is determined with a single species in a network system.

Definition 5.2. *Let $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ be a deterministic network system modeled with*

$$\frac{d}{dt}\hat{x}(t) = \hat{f}(\hat{x}(t))$$

If there exists species $S_i \in \hat{\mathcal{S}}$ such that $\{\hat{x}_1 : \hat{f}_i(x') = 0, \hat{x} = (\hat{x}_1, \ldots, \hat{x}_d) \in \mathcal{R}^d_{>0}\} = \{c\}$ for some $c > 0$, then the deterministic system is termed a S_i-definite ACR system.

Remark 5.1. *An S_i-definite ACR system is an ACR system. For a S_i-definite ACR system, an ACR species and its ACR value is solely determined by the single equation associated with the species S_i.*

A simple mass-action ACR system constructed with only two species is introduced in [5]. Let $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ be mass-action system associated with

$$Z + X_1 \xrightarrow{\theta} 2Z, \quad Z \xrightarrow{\mu} X_1. \tag{15}$$

Because any positive roots $x^* = (x_1^*, z^*)$ of the equation $\frac{d}{dt}z(t) = z(t)(\theta x_1(t) - \mu)$ for species Z satisfies $x_1^* = \frac{\mu}{\theta}$, this mass action system is an ACR system. Furthermore since we have $\{x_1 : z(\theta x_1 - \mu) = 0\} = \{\frac{\mu}{\theta}, x_1 > 0, z > 0\}$, this system is also a Z-definite ACR system by Definition 5.2. We termed this system a *basic ACR system for* X_1. This ACR system would be mainly used for control in the main text.

It is shown that there is a broad collection of networks whose associated mass-action system are ACR systems. They are characterized using network topological conditions in [5]. In the following theorem, e_i denotes a vector whose i th entry is one, and the other entries are all zeros.

Theorem 5.1 (Shinar and Feinberg 2010 [5])**.** *Let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a deficiency 1 reaction network. Suppose there are two non-terminal complexes y and $\bar{y}$ such that $y - \bar{y} = ce_i$ for some $i \in Z_{>0}$ and $c \neq 0$. Then for any set of parameters $\mathcal{K}$, the mass-action deterministic network system $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ is a mass-action ACR network system.*

The controlled deterministic system is basically a union of two deterministic systems; one is a given network system and the other is an ACR system. We formally define the union of two deterministic network systems. In the definition below, $M_{n,m}$ denote the set of all $n \times m$ matrices and I_n denotes the $n \times n$ identity matrix.

Definition 5.3. *Let $(\mathcal{S}, C\,\mathcal{R}, \mathcal{K})$ and $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ be deterministic network systems modeled with*

$$\frac{d}{dt}x(t) = f(x(t)) \quad \textit{and} \quad \frac{d}{dt}\hat{x}(t) = \hat{f}(\hat{x}(t)), \ \textit{respectively.}$$

Let $\mathcal{S} = \{X_1, \ldots, X_d, Y_1, \ldots, Y_k\}$ and $\hat{\mathcal{S}} = \{X_1, \ldots, X_{d'}, Z_1, \ldots, Z_{k'}\}$ with $d \geq d'$. Then the union system of the deterministic systems $(\mathcal{S}, C\,\mathcal{R}, \mathcal{K})$ and $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ is a deterministic system such that

$$\frac{d}{dt}\bar{x}(t) = \bar{f}(\bar{x}(t)),$$

where $\bar{x}(t) = (x_1(t), \ldots, x_d(t), y_1(t), \ldots, y_k(t), z_1(t), \ldots, z_{k'}(t))^T$ and $\bar{f} = Ef + E'f'$ with

$$E = \begin{pmatrix} I_{d+k} \\ 0 \end{pmatrix} \in M_{d+k+k',d+k}, \quad \textit{and} \quad E' = \begin{pmatrix} 0 \\ I_{d+k'} \end{pmatrix} \in M_{d+k+k',d+k'}.$$

Now, we define an ACR controller.

Definition 5.4. *Let $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ be a deterministic network system and let $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ be an ACR network system such that $X_1 \in \mathcal{S} \cap \hat{\mathcal{S}}$ and $\hat{\mathcal{S}} \setminus \mathcal{S} \neq \emptyset$. If the union of the two network systems is an ACR network system such that X_1 is an ACR species, then $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ is termed an ACR controller for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the union system is called a controlled system. Furthermore, if ACR controller $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ is a mass-action system, then it is termed a mass-action ACR controller for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$.*

6 Steady states and stability using an ACR controller

In this section, we show that for any deterministic system modeled with general kinetics, an ACR controller endows ACR to the given system and drives the long-term behavior of a target species towards the desired value. For the basic ACR controller, the existence of the steady states will now be verified together with their stability.

Lemma 6.1. *Let $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ be a deterministic network system such that $X_1 \in \mathcal{S}$. Let $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ be a Z-definite ACR system such that $X_1 \in \hat{\mathcal{S}}$ and $Z \notin \mathcal{S}$. If the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ admits a positive steady state, then it is an ACR system, and X_1 is an ACR species.*

Proof. Since $Z \notin S$, the equation $\frac{d}{dt}z(t) = 0$ for Z in the union system is same as the equation for Z in ACR system $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$. At the expense of abusing the notation, we let $\frac{d}{dt}z(t) = f_z(x(t))$ and $\frac{d}{dt}\bar{z}(t) = f_z(\bar{x}(t))$ be the equations for Z in $(\hat{\mathcal{S}}, \hat{\mathcal{C}}, \hat{\mathcal{R}}, \hat{\mathcal{K}})$ and the union system, respectively. By definition of the Z-definite ACR system, if $f_z(x) = 0$ then there exists a positive real number c such that $x_1 = c$. Hence, for each positive steady state $\bar{x}^*$ in the union system, $\bar{x}_1^* = c$ and therefore X_1 is an ACR species in the union system. □

For a given network system $(\mathcal{S}, \mathcal{C}, \mathcal{R})$, suppose $Z \notin \mathcal{S}$ and $X_1 \in \mathcal{S}$. Since the basic ACR controller (15) for X_1 is a Z-definite ACR system, it is a mass action ACR controller for $(\mathcal{S}, \mathcal{C}, \mathcal{R})$. We call this basic ACR system the *basic ACR controller* interchangeably.

Lemma 6.1 guarantees that the values of X_1 must be c at any positive steady states as long as a positive steady state exists in the union system. The following theorem provides a sufficient condition of a given network system $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ for existence of a positive steady state in the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the basic ACR controller.

Lemma 6.2. *Let $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ be a deterministic network system modeled with*

$$\frac{d}{dt}x(t) = f(x(t)).$$

Let $PS = \{x : x = (x_1, x_2, \ldots, x_d) \in \mathcal{R}^d_{>0}, f(x) = 0\}$. Suppose there exists an $x^* \in PS$ such that $x_1^* = \frac{\mu}{\theta}$, then the basic ACR network system (15) is a mass-action ACR controller for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$, and the controlled system admits a positive steady state.

In the following proof, the concatenation $w = (u, v)$ for $u \in \mathbb{R}^d$ and $b \in \mathbb{R}^1$ denotes a vector in $\mathbb{R}^{d+1}$ such that $w_i = u_i$ for $i = 1, 2, \ldots, d$ and $w_{d+1} = v$.

Proof. Let $\mathcal{S} = \{X_1, \ldots, X_d\}$. Let $\bar{x} = (x, z)$ for each $x \in \mathbb{R}^d_{\geq 0}$ and $z \in \mathbb{R}_{\geq 0}$ be a solution to the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the basic ACR network system (15). Then $\bar{x}$ satisfies

$$\frac{d}{dt}\bar{x}(t) = \bar{f}(\bar{x}(t)), \tag{16}$$

for some $\bar{f}$. By the construction of the union system, we have

$$\bar{f}_i(\bar{x}) = \begin{cases} f_i(x) - z(\theta x_1 - \mu) & \text{if } i = 1, \\ z(\theta x_1 - \mu) & \text{if } i = d+1, \\ f_i(x), & \text{otherwise.} \end{cases} \tag{17}$$

Let x^* be a positive steady state of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ such that $x_1 = \frac{\mu}{\theta}$. For any positive value z^*, we have $\bar{f}(\bar{x}^*) = 0$ where $\bar{x}^* = (x^*, z^*)$. □

The convergence to positive steady states in general controlled systems with a ACR controller is more delicate problem since the actual network structure and parameters need probably to be specified. However, if linear stability condition is held for a given system as well as the conditions in Lemma 6.2 with some additional conditions, then the controlled system with the basic ACR system (15) admits linear stability. Linear stability of a steady state holds if each eigenvalues of the Jacobian of a dynamical system at the steady state has a strictly negative real part. This implies the dynamical system asymptotically converges to the steady state if its initial state was close enough to the steady state.

Remark that in case a given system has no conservation relation, the dynamics is not confined into a lower dimensional stoichiometry class. Hence if we assume linear stability of the given system at a positive steady state x^*, all eigenvalues of the Jacobian at the steady state have strictly negative real parts. Hence we can maintain the linear stability after we add a ACR controller if the parameters of the ACR controller are small enough. This is by the fact that the roots of the characteristic polynomial are continuous with respect to the coefficients, hence the eigenvalues of the Jacobian of the controlled system still have strictly negative real parts.

Hence we investigate the stability of the controlled system when a given system $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ admits conservation relations. Let $x(t) = (x_1(t), \dots, x_d(t))$ be the deterministic model associated with $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ such as (11) in $\mathbb{R}^d_{>0}$. Suppose that $v^1, \dots, v^k$ are positive vectors such that $v^i \cdot \frac{d}{dt}x(t) = 0$ for all t and for each i, where $\cdot$ means the canonical inner product between two finite dimensional euclidean vectors. This implies that for a fixed initial state $x(0)$, there exist M_i's such that

$$u^i \cdot x(t) = M_i \quad \text{for all } t.$$

Without loss of generality, we suppose u^i are linear independent. Then in the following way, we can reduce the system onto a lower dimension system that admits no conservative relations. First note that since we assume the linear independence of u^i's, we have $k \leq d$. Hence using Gaussian elimination and by rearranging the coordinate of x, we have

$$\left[U|I\right] \begin{bmatrix} x_1(t) \\ x_2(t) \\ \vdots \\ x_{\bar{d}}(t) \\ x_{\bar{d}+1}(t) \\ x_d(t) \end{bmatrix} = \begin{bmatrix} M_1 \\ M_2 \\ \vdots \\ M_k \end{bmatrix}, \tag{18}$$

where $\bar{d} = d - k$, the matrix I is the k dimensional identity matrix, U is some $\bar{d} \times k$ matrix and

M_i's are some constants. Hence we have

$$\begin{aligned} x_{\bar{d}+1}(t) &= M_1 - \sum_{i=1}^{\bar{d}} u_{1i} x_i(t), \\ x_{\bar{d}+2}(t) &= M_2 - \sum_{i=1}^{\bar{d}} u_{1i} x_i(t), \\ &\vdots \\ x_d(t) &= M_k - \sum_{i=1}^{\bar{d}} u_{1i} x_i(t). \end{aligned} \tag{19}$$

This implies the variables $x_{\bar{d}+1}, \ldots, x_d$ are completely determined by relations (19). Then we have the following reduced system,

$$\begin{aligned} &\frac{d}{dt} x_i(t) = g_i(x_1(t), x_2(t), \ldots, x_{\bar{d}}(t)) \quad \text{where,} \\ &g_i(x_1, x_2, \ldots, x_{\bar{d}}) = f_i \left(x_1, x_2, \ldots, x_{\bar{d}}, M_1 - \sum_{i=1}^{\bar{d}} u_{1i} x_i(t), \ldots, M_k - \sum_{i=1}^{\bar{d}} u_{ki} x_i(t) \right). \end{aligned} \tag{20}$$

for $i = 1, 2, \ldots, \bar{d}$. Note that this reduced system is specified with the choice of initial state $x(0)$ as the initial condition determines the conservative quantity M_i's. Note further that since the steady state values of $x_{\bar{d}+i}$ for $i = 1, 2 \ldots, k$ are completely determined by the steady state values x_i for $i = 1, 2, \ldots, \bar{d}$, the stability of $x(t)$ is also determined by the reduced system $(x_1(t), \ldots, x_{\bar{d}}(t))$. The linear stability of the reduced system is investigated with the eigenvalues of Jacobian. We denote $J(x^*)$ be the Jacobian of this reduced system at x^*, where we abuse the notation since x^* is a state in the original system but the Jacobian is for the reduced system.

Now we suppose that species X_1 is the control target with the basic ACR controller

$$X_1 + Z \xrightarrow{\theta} 2Z, \quad Z \xrightarrow{\mu} X_1. \tag{21}$$

Suppose, without loss of generality, $u_{11} \neq 0$. That means S_1 is involved at least one conservative relation. Then we have new conservation relations in the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the basic ACR system. We let

$$\bar{M}_i = \begin{cases} v^i \cdot x(0) + z(0) = M_i + z(0) & \text{if } v_1 \neq 0, \\ v^i \cdot x(0) + z(0) = M_i, & \text{otherwise.} \end{cases} \tag{22}$$

Then the new conservative relations are represented as

$$\left[U \mid u_1 \mid I \right] \begin{bmatrix} x_1(t) \\ x_2(t) \\ \vdots \\ x_{\bar{d}}(t) \\ z(t) \\ x_{\bar{d}+1}(t) \\ x_d(t) \end{bmatrix} = \begin{bmatrix} \bar{M}_1 \\ \bar{M}_2 \\ \vdots \\ \bar{M}_k \end{bmatrix}, \tag{23}$$

where v is the first column vector of U, and U and I are the same matrices as (18). The definition of u_1 basically means that the control species Z is involved in the same conservation relation as X_1 in the original system $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$. Hence the dynamics $\bar{x}(t) = (\bar{x}_1(t), \ldots, \bar{x}_{\bar{d}}(t), z(t))$ associated with the union system can also be reduced to

$$\frac{d}{dt}\bar{x}_i(t) = h_i(x_1(t), x_2(t), \ldots, x_{\bar{d}}(t), z(t)). \tag{24}$$

where,

$$h_i(x_1, x_2, \ldots, x_{\bar{d}}, z) =$$
$$\begin{cases} f_1\left(x_1, x_2, \ldots, x_{\bar{d}}, \bar{M}_1 - \sum_i^{i=\bar{d}} u_{1i}x_i(t) - v_1 z(t), , \ldots, \bar{M}_k - \sum_i^{i=\bar{d}} u_{ki}x_i(t) - v_k z(t)\right) - z(\theta x_1 - \mu), & \\ & \text{if } i = 1, \\ z(\theta x_1 - \mu) & \text{if } i = \bar{d}+1, \\ f_i\left(x_1, x_2, \ldots, x_{\bar{d}}, \bar{M}_1 - \sum_i^{i=\bar{d}} u_{1i}x_i(t) - v_1 z(t), , \ldots, \bar{M}_k - \sum_i^{i=\bar{d}} u_{ki}x_i(t) - v_k z(t)\right), & \text{otherwise} \end{cases}$$

Note that by (22), we have $\partial_i h_j(x_1^*, \ldots, x_{\bar{d}}^*) = \partial_i g_j(x_1^*, \ldots, x_{\bar{d}}^*)$ for $i, j \in \{1, 2, \ldots, \bar{d}\}$. Then the jacobian $\bar{J}(x^*, z^*)$ for this system at (x^*, z^*) where $x_1^* = \frac{\mu}{\theta}$ is

$$\bar{J}(x^*, z^*) =$$
$$\begin{bmatrix} \partial_1 h_1(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_2 h_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \partial_{\bar{d}} h_1(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*) \\ \partial_1 h_2(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_2 h_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \partial_{\bar{d}} h_2(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_z h_2(x_1^*, \ldots, x_{\bar{d}}^*) \\ & & \vdots & & \\ \partial_1 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \partial_{\bar{d}} h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_z h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) \\ \theta z^* & 0 & \cdots & 0 & 0 \end{bmatrix}$$
$$= \begin{bmatrix} \partial_1 g_1(x_1^*, \ldots, x_{\bar{d}}^*) - \theta z^* & \partial_2 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \partial_{\bar{d}} g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*) \\ \partial_1 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*)) \\ & & \vdots & & \\ \partial_1 g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \partial_z h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*)) \\ \theta z^* & 0 & \cdots & 0 & 0 \end{bmatrix} \tag{25}$$

Note that by the chain rule, the entries of the last column follow

$$\partial_z h_i(x_1^*, \ldots, x_{\bar{d}}^*)$$
$$= -\sum_{i=1}^{k} \partial_{\bar{d}+i} f_i \left(x_1^*, x_2^*, \ldots, x_{\bar{d}}^*, M_1 - \sum_i^{i=\bar{d}} u_{1i}x_i^*(t) - e_i z(t), , \ldots, M_k - \sum_i^{i=\bar{d}} u_{ki}x_i^*(t) - v_k z(t)\right)$$
$$= -\sum_{i=1}^{k} v_i \partial_{\bar{d}+i} f_i(x^*, z^*) \quad \text{for } i = 2, 3, \ldots, \bar{d},$$

and similarly

$$\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*))$$
$$\left(-\sum_{i=1}^{k} v_i \partial_{\bar{d}+i} f_1(x, z) - (\theta x_1 - \mu)\right)\Big|_{x=x^*, z=z^*} = -\sum_{i=1}^{k} v_i \partial_{\bar{d}+i} f_1(x^*, z^*) \quad \text{since } x_1^* = \frac{\mu}{\theta}.$$

As the stability of $x(t)$ is determined by the stability of its reduced system, we consider the linear stability of the reduced system (24) of $\bar{x}(t)$ to study the stability of the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the basic ACR controller (21). For the linear stability, we show that the characteristic function for $\bar{J}(x^*, z^*)$ will be the characteristic function of $J(x^*)$ with some perturbation. To show this, we use the conventional notation for deterministic $|A|$ for a square matrix A. I denotes an identity matrix, and it could denote a different dimensional identity matrix according to the content. We will also use the column/row expansion of deterministic. We further also use the row decomposition for determinant. The row decomposition of the determinant means that when A is a square matrix such that the first row A_1 is equal to $A_1' + A_1''$ with some row vectors A_1' and A_1'', we have $|A| = |A'| + |A''|$ where A' and A'' are square matrices whose first row is replaced with A_1' and A_1'', respectively.

$$
\begin{aligned}
&|\lambda I - \bar{J}(x^*, z^*)| = \\
&\begin{vmatrix}
\lambda - \partial_1 g_1(x_1^*, \ldots, x_{\bar{d}}^*) + \theta z^* & -\partial_2 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_1(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*) \\
-\partial_1 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \lambda - \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*)) \\
 & & \vdots & & \\
-\partial_1 g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \lambda - \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*)) \\
-\theta z^* & 0 & \cdots & 0 & \lambda
\end{vmatrix} \\
&= \lambda \begin{vmatrix}
\lambda - \partial_1 g_1(x_1^*, \ldots, x_{\bar{d}}^*) + \theta z^* & -\partial_2 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_1(x_1^*, \ldots, x_{\bar{d}}^*) \\
-\partial_1 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \lambda - \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) \\
 & \vdots & & \\
-\partial_1 g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \lambda - \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*)
\end{vmatrix} \\
&- (-1)^{\bar{d}+2} \theta z^* \begin{vmatrix}
-\partial_2 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_1(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*) \\
\lambda - \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*)) \\
 & \vdots & & \\
-\partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \lambda - \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*))
\end{vmatrix} \\
&= \lambda \begin{vmatrix}
\lambda - \partial_1 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_2 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_1(x_1^*, \ldots, x_{\bar{d}}^*) \\
-\partial_1 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \lambda - \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) \\
 & \vdots & & \\
-\partial_1 g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \lambda - \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*)
\end{vmatrix} \\
&+ \theta z^* \lambda \begin{vmatrix}
1 & 0 & \cdots & 0 \\
-\partial_1 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \lambda - \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) \\
 & \vdots & & \\
-\partial_1 g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \lambda - \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*)
\end{vmatrix} \\
&- (-1)^{\bar{d}+2} \theta z^* \begin{vmatrix}
-\partial_2 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_1(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*) \\
\lambda - \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*)) \\
 & \vdots & & \\
-\partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \lambda - \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*))
\end{vmatrix}
\end{aligned}
\tag{26}
$$

Notice that the first term in (26) is equal to $\lambda|\lambda I - J(x^*)|$. We denote $\lambda G(\lambda), \theta z^* \lambda H_1(\lambda)$ and

$(-1)^{\bar{d}+2}\theta z^* H_2(\lambda)$ the first, the second and the third term in (26), respectively. Hence we have

$$|\lambda I - \bar{J}(x^*, z^*)| = \lambda G(\lambda) + \theta z^* \lambda H_1(\lambda) - (-1)^{\bar{d}+2}\theta z^* H_2(\lambda). \tag{27}$$

Now, using the same notations above, we state a theorem related to the stability of (x^*, z^*) of the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the basic ACR system (21).

Theorem 6.3. *Suppose the conditions in Lemma 6.2 hold. Suppose further*

1. *the associated system for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ admits conservative relations such as (18) and $u_{11} \neq 0$,*
2. *all the eigenvalues of $J(x^*)$ have strictly negative real parts, and*
3. *$H_2(0) > 0$ if $\bar{d}$ is odd and $H_2(0) < 0$ if $\bar{d}$ is even.*

Then for sufficiently small θ and μ and for sufficiently large $z(0)$, all the eigenvalues of $\bar{J}(x^*, z^*)$ have also strictly negative real parts. That is the positive steady state (x^*, z^*) is linear stable in the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the basic ACR system (21).

Proof. First of all, we scale $\mu = \epsilon^2\bar{\mu}, \theta = \epsilon^2\bar{\theta}$ and $z(0) = \frac{M}{\epsilon}$ for some M. Note that $v_1 = u_{11} \neq 0$ by hypothesis 1 in the statement. Then by (22) and (23), we have

$$\begin{aligned} z^* &= \bar{M}_1 - x_1^* - \sum_{j=2}^{\bar{d}} \frac{u_{1j}}{v_1} x_j^* \\ &= \bar{M}_1 - \frac{\mu}{\theta} - \sum_{j=2}^{\bar{d}} \frac{u_{1j}}{v_1} x_j^* \\ &= z(0) + \sum_{j=1}^{\bar{d}} \frac{u_{1j}}{v_1} x_j(0) - \frac{\mu}{\theta} - \sum_{j=2}^{\bar{d}} \frac{u_{1j}}{v_1} x_j^*, \end{aligned}$$

for each i such that $u_{i1} \neq 0$. Thus for each ϵ, we have

$$\theta z^* = \epsilon\bar{\theta}\left(M + \epsilon \sum_{j=1}^{\bar{d}} \frac{u_{ij}}{v_1} x_j(0) - \epsilon\frac{\mu}{\theta} - \epsilon \sum_{j=2}^{\bar{d}} \frac{u_{ij}}{v_1} x_j^*\right) > 0$$

by taking sufficiently large $M = M(\epsilon)$. We denote $c(\epsilon) = \theta z^*$, then $\lim_{\epsilon\to 0} c(\epsilon) = 0$.

By the hypothesis, all roots of $G(\lambda)$ have strictly negative real parts. Let $\lambda_0, \lambda_1, \ldots, \lambda_{\bar{d}}$ be the roots of $\lambda G(\lambda)$ where $\lambda_0 = 0$ and λ_i are non-zero roots with strictly negative real parts. Let further denote $\lambda_0(\epsilon), \lambda_1(\epsilon), \ldots, \lambda_{\bar{d}}(\epsilon)$ the roots of $|\lambda I - \bar{J}(x^*, z^*)| = \lambda G(\lambda) + c(\epsilon)\lambda H_1(\lambda) - (-1)^{\bar{d}+2} c(\epsilon) H_2(\lambda)$. By the continuity of roots of a polynomial with respect to the coefficients, we have $\lim_{\epsilon\to 0} |\lambda_i(\epsilon) - \lambda_i| = 0$ for $i = 1, 2, \ldots, \bar{d}$. Hence with small enough ϵ, we could make the real parts of $\lambda_i(\epsilon)$ is still negative for each $i = 1, 2 \ldots, \bar{d}$.

We turn to show that $\lambda_0(\epsilon)$ has also a strictly negative real part. Note that $|\lambda I - \bar{J}(x^*, z^*)|\Big|_{\lambda=0} = (\lambda - \lambda_0(\epsilon))(\lambda - \lambda_1(\epsilon)) \cdots (\lambda - \lambda_{\bar{d}}(\epsilon))$. Hence

$$(-1)^{\bar{d}+1}\lambda_0(\epsilon)\lambda_1(\epsilon)\cdots\lambda_{\bar{d}}(\epsilon) = -(-1)^{\bar{d}+2} c(\epsilon) H_2(0). \tag{28}$$

Suppose $\lambda_0(\epsilon)$ is a complex number with non-zero imaginary part. Then it must be a conjugate of $\lambda_i(\epsilon)$ for some i. Since we choose ϵ small enough so that the real part of each $\lambda_i(\epsilon)$ is strictly negative, $\lambda_0(\epsilon)$ has a negative real part. Now we suppose that $\lambda_0(\epsilon)$ is a real number. In this case, because of the negative real parts of $\lambda_i(\epsilon)$, the product $\prod_{i=1}^{\bar{d}} \lambda_i(\epsilon)$ is negative if $\bar{d}$ is odd and is positive otherwise. Thus by the hypothesis 2 and (28), we have $\lambda_0(\epsilon) < 0$. Thus the result follows. □

7 Control of networks with no positive steady states

In this section, we introduce an ACR system that controls both a target species and other species in a given network system. By using this type of expanded ACR system, we show that a 2-dimensional reaction system, which admits no positive steady states, can be controlled as we showed in Section 2.3 of the main text. For a two dimensional system with species A and B, let A be the target species we desire to control. Then we define a mass action ACR system (29) that controls the other species B as well as the target species A,

$$A + Z \xrightarrow{\theta} 2Z, \quad Z \xrightarrow{\mu} A, \quad B + Z \underset{\alpha_2}{\overset{\alpha_1}{\rightleftharpoons}} Z. \tag{29}$$

Lemma 7.1. *Let $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ be a network system such that $\mathcal{S} = \{A, B\}$. Let $x(t) = (a(t), b(t))$ be the associated deterministic system such that*

$$\frac{d}{dt}a(t) = f_1(a(t), b(t)), \quad \textit{and} \quad \frac{d}{dt}b(t) = f_2(a(t), b(t)).$$

Suppose that there is no positive steady state for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$. Suppose further that for any positive constant c, there exists $d > 0$ such that $f_1(c, d) = 0$. Then the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and (29) admits a positive steady state (a^*, b^*, z^*) such that $a^* = \frac{\mu}{\theta}$ for any μ and θ provided $\alpha_1 b^* > \alpha_2$ when $f_2(\frac{\mu}{\theta}, b^*) > 0$ and $\alpha_1 b^* < \alpha_2$ when $f_2(\frac{\mu}{\theta}, b^*) < 0$.

Remark 7.1. *In the following proof, it is shown that b^* is independent of α_1 and α_2. Hence we can evaluate the value of $f_2(\frac{\mu}{\theta}, b^*)$ and then we set the parameters α_1 and α_2 in the controller (29) according to the sign of $f_2(\frac{\mu}{\theta}, b^*)$.*

Proof. First, the reactions $B + Z \rightleftharpoons Z$ do not change the concentration of Z. Hence A is still an ACR species as we showed around (15). Let $\bar{x} = (\bar{a}, \bar{b}, z)$ be the solution to the deterministic system associated with the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and (29). Then we have

$$\begin{aligned}
&\frac{d}{dt}\bar{a}(t) = \bar{f}_1(\bar{a}, \bar{b}, z) = f_1(\bar{a}, \bar{b}) - z(\theta\bar{a} - \mu),\\
&\frac{d}{dt}\bar{b}(t) = \bar{f}_2(\bar{a}, \bar{b}, z) = f_2(\bar{a}, \bar{b}) - z(\alpha_1\bar{b} - \alpha_2), \text{ and}\\
&\frac{d}{dt}z(t) = z(\theta\bar{a} - \mu).
\end{aligned}$$

By the hypothesis, there exists a positive constant x_2^* such that $\bar{f}_1(\frac{\mu}{\theta}, x_2^*, z) = f_1(\frac{\mu}{\theta}, x_2^*) = 0$ for any z. Then if we choose $z^* = \dfrac{f_2(\frac{\mu}{\theta}, x_2^*)}{\alpha_1 x_2^* - \alpha_2}$, we have $\bar{f}_2(\frac{\mu}{\theta}, x_2^*, z^*) = 0$. Since $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ does not admits a positive steady state, $f_2(\frac{\mu}{\theta}, x_2^*) \neq 0$. Thus $z^* \neq 0$.

It follows that $z^* > 0$ because we assumed that $\alpha_1 x_2^* > \alpha_2$ if $f_2(\frac{\mu}{\theta}, x_2^*) > 0$ and $\alpha_1 x_2^* < \alpha_2$ if $f_2(\frac{\mu}{\theta}, x_2^*) < 0$. Therefore $(\frac{\mu}{\theta}, x_2^*, z^*)$ is a positive steady state of the union system. □

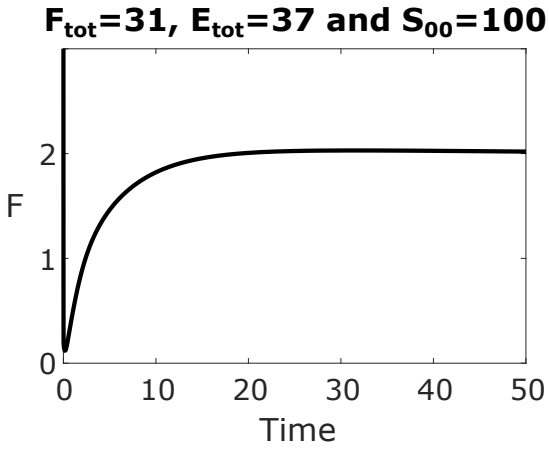
8 Applications of the deterministic results

8.1 ERK system

In this section, we analyze the stability of the controlled ERK system shown in Figure 2 a. We show that both the conditions in Lemma 6.2 and conditions in Theorem 6.3 hold for a positive steady state (x^*, z^*) in the controlled ERK system. Let $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ be the deterministic system associated with the ERK network in Figure 2 a using the parameters given in the main text. Let also $(\bar{\mathcal{S}}, \bar{\mathcal{C}}, \bar{\mathcal{R}}, \bar{\mathcal{K}})$ be the union system of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and the ACR controller in 2 a with $\theta = 1$ and $\mu = 2$. We use a Matlab simulation to obtain a positive steady state, as well as the relevant Jacobian, eigenvalues and determinant.

Let $x(t) = (x_1(t), x_2(t), \ldots, x_{12}(t))$ and $\bar{x}(t) = (\bar{x}_1(t), \bar{x}_2(t), \ldots, \bar{x}_{12}(t), z(t))$ represent the concentrations of species in the systems $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ and $(\bar{\mathcal{S}}, \bar{\mathcal{C}}, \bar{\mathcal{R}}, \bar{\mathcal{K}})$, respectively. We arrange $x_1, x_2, \ldots, x_{12}$ so that they represent $F, E, S_{00}, S_{01}, S_{10}, ES_{00}, ES_{01}, FS_{01}, FS_{10}, S_{11}, ES_{10}$ and FS_{11}, respectively. We also let $\bar{x}_i$ represent the same concentration as x_i, and we let z represent the concentration of Z.

To show the condition in Lemma 6.2, we show that the system $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ admits a positive steady state at x^* such that $x_1^* = \frac{\mu}{\theta} = 2$. We verify the existence of the positive steady state using the simulation shown in the figure below for the ERK system with $F_{\text{tot}} = 31, E_{\text{tot}} = 37, S_{\text{tot}} = 100$.



The positive steady state $x^* = (2.0, 2.1, 7.8, 0.9, 11.9, 16.7, 7.6, 1.5, 15.2, 15.3, 10.9, 12.3)$. We rounded off the values to one decimal place.

We also notice there are three linear independent conservative relations in $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$,

$$\begin{aligned} x_{12}(t) &= F_{\text{tot}} - x_1(t) - x_8(t) - x_9(t), \\ x_{11}(t) &= E_{\text{tot}} - x_2(t) - x_6(t) - x_7(t), \\ x_{10}(t) &= S_{\text{tot}} - \sum_{i=3}^{12} x_i(t). \end{aligned} \tag{30}$$

The target species F is involved in the first conservative relation in (30). Hence condition 1 in Theorem 6.3 holds.

For the second condition in Theorem 6.3, we have the following Jacobian $J(x^*)$ of the reduced system obtained by using the conservation laws (30) for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ as (19).

$$J(x^*) = \begin{bmatrix} -25.3 & -3.0 & 3.0 & -3.0 & 1.5 & 0 & 0 & 3.0 & 2.0 \\ 0 & -90.8 & -1.0 & -1.0 * -1.0 & -5.0 & -4.0 & 0 & 0 & \\ 0 & -56.2 & -1.0 & 0 & 0 & 2.0 & 0 & 1.0 & 1.0 \\ -4.4 & -3.3 & 0 - 7.0 & 0 & 0 & 1.0 & 4.0 & 0 & \\ -8.1 & -29.2 & 0 & 0 & -2.5 & -5.0 & -5.0 & 0 & 3.0 \\ 0 & 56.2 & 1.0 & 0 & 0 & -3.0 & 0 & 0 & 0 \\ 0 & 3.3 & 0 & 1.0 & 0 & 1.0 & -3.0 & 0 & 0 \\ 4.4 & 0 & 0 & 6.0 & 0 & 0 & 0 & -5.0 & 0 \\ 5.1 & 0 & 0 & 0 & 1.5 & 0 & 0 & -3.0 & -7.0 \end{bmatrix} \tag{31}$$

The eigenvalues of $J(x^*)$ are $-0.11, -0.8, -1.4, -4.1, -6.0, -7.5, -9.3, -27.0, -88.2$.

For the third condition in Theorem 6.3, we note that $d = 12, k = 3$ and hence $\bar{d} = 9$. Thus, if $H_2(0) > 0$, then the condition holds. Note that we have three conservation relations for the system $(\bar{\mathcal{S}}, \bar{\mathcal{C}}, \bar{\mathcal{R}}, \bar{\mathcal{K}})$

$$\begin{aligned} x_{12}(t) &= F_{\text{tot}} - x_1(t) - x_8(t) - x_9(t) - z(t), \\ x_{11}(t) &= E_{\text{tot}} - x_2(t) - x_6(t) - x_7(t), \\ x_{10}(t) &= S_{\text{tot}} - \sum_{i=3}^{12} x_i(t). \end{aligned}$$

Hence using the same notation in Section 6, we have

$$\partial_z h_i(x_1^*, \ldots, x_{\bar{d}}^*) = \begin{cases} -2 & \text{if } i = 1, \\ -3 & \text{if } i = 9, \text{ and} \\ 0 & \text{otherwise.} \end{cases}$$

Combining this with (31), we obtain the matrix

$$\begin{pmatrix} -\partial_2 g_1(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_1(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*) \\ \lambda - \partial_2 g_2(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & -\partial_{\bar{d}} g_2(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_1(x_1^*, \ldots, x_{\bar{d}}^*)) \\ & \vdots & & \\ -\partial_2 h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & \cdots & \lambda - \partial_{\bar{d}} g_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*) & -\partial_z h_{\bar{d}}(x_1^*, \ldots, x_{\bar{d}}^*)) \end{pmatrix}$$

shown in (26). By plugging in $\lambda = 0$ into the matrix and computing its determinant, we have $H_2(0) = 3.3 \times 10^6$. Since $\bar{d} = 9$, the third condition hold in Lemma 6.2.

Consequently we show that all the conditions in Lemma 6.2 and show that Theorem hold 6.3, hence the linear stability of $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ follows.

8.2 A 2-dimensional system admitting no positive steady states

In this section, we use the expanded ACR controller (29) to control the 2-dimensional system

$$A+B \xrightarrow{1} \emptyset \begin{array}{l} \xrightarrow{3} A \\ \xrightarrow{5} B, \end{array} \tag{32}$$

which is introduced in Section 2.4 of the main text. The mass-action deterministic system associated with this network is

$$\begin{aligned} \frac{d}{dt}a(t) &= f_1(a,b) = -a(t)b(t)+3, \\ \frac{d}{dt}b(t) &= f_2(a,b) = -a(t)b(t)+5. \end{aligned}$$

Note that this system does not admit a positive steady state, as there does not exists $(a^*,b^*)\in\mathbb{R}^2_{>0}$ such that $3-a^*b^*=0$ and $5-a^*b^*=0$. In particular, $\lim_{t\to\infty}(b(t)-a(t))=\infty$ since $\frac{d}{dt}(b(t)-a(t))=2$.

Furthermore the union system of (32) and the basic ACR system $Z+A\xrightarrow{1}2Z$ and $Z\xrightarrow{5}A$ also does not admit a positive steady state. The associated mass-action system is

$$\begin{aligned} \frac{d}{dt}a(t) &= -a(t)b(t)+3-z(t)(a(t)-5), \\ \frac{d}{dt}b(t) &= -a(t)b(t)+5, \\ \frac{d}{dt}z(t) &= z(t)(a(t)-5). \end{aligned}$$

Suppose there exists a positive steady state (a^*,b^*,z^*). By the last equation, $a^*=5$. Plugging $a^*=5$ into $a(t)$ in the first equation, we have $b^*=\frac{3}{5}$. However, at this state, b is not stabilized as $-a^*b^*+5=2$, hence it contradicts to the assumption that (a^*,b^*,z^*) is a positive steady state.

Now we consider the union system of (32) and the expanded ACR controller (29) introduced in Section 7.

$$Z+A\xrightarrow{\theta}2Z,\quad Z\xrightarrow{\mu}A,\quad Z+B\underset{\alpha_2}{\overset{\alpha_1}{\rightleftharpoons}}Z,$$

with general positive parameters $\kappa_1,\kappa_2,\kappa_3,\theta,\mu,\alpha_1$ and α_2. We use Lemma 7.1 to show this union system admits a positive steady state under a mile condition. The associated mass-action system is

$$\begin{aligned} \frac{d}{dt}a(t) &= -a(t)b(t)+3-z(t)(\theta a(t)-\mu), \\ \frac{d}{dt}b(t) &= -a(t)b(t)+5-z(t)(\alpha_1 b(t)-\alpha_2), \\ \frac{d}{dt}z(t) &= z(t)(\theta a(t)-\mu). \end{aligned}$$

It can be easily shown that for any positive constant c, there exists $d = \frac{3}{d}$ such that $f_1(c,d) = 0$. Hence for $a^* = \frac{\mu}{\theta}$ with arbitrary $\mu > 0$ and $\theta > 0$, there exists $b^* = \frac{3\theta}{\mu}$ such that $f_1(a^*, b^*) = 0$. Since $f_2(a^*, b^*) = 2 > 0$, we tune the parameters $\alpha_1 > 0$ and $\alpha_2 > 0$ in (29) as they satisfy $\alpha_1 b^* > \alpha_2$.

Hence by Lemma 7.1,

$$(a^*, b^*, z^*) = \left(\frac{\mu}{\theta}, \frac{\kappa_2 \theta}{\kappa_1 \mu}, \frac{\kappa_1 \mu(\kappa_3 - \kappa_2)}{\alpha_1 \kappa_2 \theta - \alpha_2 \kappa_1 \mu} \right)$$

is a positive steady state.

9 Stochastic ACR control

To control a stochastic system via an ACR controller, we rely on an approximation under multi-scaling model reduction as described in Section 2.4 and 2.5 of the main text. In this section we introduce the formal procedures for generating a reduced model, and we introduce related theorems.

9.1 Network reduction

To formally define a reduced model of a given stochastic system, a notion of network projection needs to be introduced. The reduced system shown in Figure 4 b and the hybrid system shown in 6 d are obtained through network projection. For example, consider the reaction network

$$A \underset{\kappa_1}{\overset{\kappa_2}{\rightleftharpoons}} B. \tag{33}$$

Suppose that for some parameters, species B is rarely produced or removed until time $t = 1$. In this case, we approximate the distribution of A with the stochastic system associated with

$$A \underset{\kappa_1}{\overset{\kappa_2 B(0)}{\rightleftharpoons}} 0. \tag{34}$$

Note that this reduced network is obtained by freezing the copy number of B at $B(0)$. We call network (34) the projection of (33) by freezing species B at $B(0)$. As this example shows, network projection can be used to describe an asymptotic behavior of a subset of species.

We define a projection function for complexes and reactions in $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ with $\mathcal{S} = \mathcal{S}_L \cup \mathcal{S}_H$ where $\mathcal{S}_L = \{S_1, S_2, \ldots, S_d\}$ and $\mathcal{S}_H = \{S_{d+1}, S_{d+2}, \ldots, S_{d+r}\}$. In the later section, $\mathcal{S}_L$ and $\mathcal{S}_H$ would represent collections of species with low and high copy numbers, respectively. Let $q_L : \mathbb{Z}^{d+r} \to \mathbb{Z}^d$ and $q_H : \mathbb{Z}^{d+r} \to \mathbb{Z}^r$ be projection function such that for each $v = (v_1, \ldots, v_d, v_{d+1}, \ldots, v_{d+r})^T \in \mathbb{Z}^{d+r}$,

$$q_L(v) = (v_1, v_2, \ldots, v_d)^T \in \mathbb{Z}^d \quad \text{and} \quad q_H(v) = (v_{d+1}, v_{d+2}, \ldots, v_{d+r})^T \in \mathbb{Z}^r.$$

We use the projection function q_L and q_H for complexes and reaction. For example, for a network $A + B \to B$ with complexes A and B, we let $\mathcal{S}_L = \{A\}$. Then the complex A, B and the

reaction $A + B \to B$ are represented with two dimensional vectors $(1,1)^T, (0,1)^T$ and $(-1,0)$, respectively. Then the projection of A, B and the reaction $A + B \to B$ are $q_L((1,1)^T) = 1$, $q_L((0,1)^T) = 0$ and $q_L((-1,0)^T) = -1$ which are associated with complexes A, 0 and reaction $A \to 0$, respectively. Hence by abusing notation, $q_L(A) = A, q_L(B) = 0$ and $q_L(A + B \to B) = A \to 0$. Generally, we denote $q_L(y)$ the complex obtained by projection of the complex vector associated with a complex y. In this way, the projected network $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L)$ of the original reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ by q_L is defined to be

$$\begin{aligned} &\mathcal{S}_L = \{S_1, \ldots, S_d\},\ \mathcal{C}_L = \{q_L(y) : y \in \mathcal{C}\},\ \text{and} \\ &\mathcal{R}_L = \{q_L(y) \to q_L(y') : y \to y' \in \mathcal{R}\ \text{ such that } q_L(y') - q_L(y) \neq \overrightarrow{0}\}. \end{aligned} \tag{35}$$

The rate constants of the projected network are defined with a given rate constants $\mathcal{K}$ of a given network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$. We inherit $\mathcal{K}$ to the projected network by incorporating the terms coming from freezing species $\mathcal{S}_H$ at their initial count. For example, the rate constant of reaction $A \to 0$ in (34) is $\kappa_2 B(0)$, where κ_2 is inherited from reaction $A + B \to B$.

Hence for the set of reaction intensities $\mathcal{K}$ in a given network $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$, the set of reaction intensities for a projected network is

$$\mathcal{K}_L = \left\{ \bar{\lambda}_u(x) = \sum_{\substack{y_k \to y_k' \in \mathcal{R} \\ q_L(y_k) = \bar{y}_u, q_L(y_k') = \bar{y}_u'}} q_H(X(0))^{q_H(y)} \lambda_k(x) : \bar{y}_u \to \bar{y}_u' \in \mathcal{R}_L \right\}, \tag{36}$$

where $u^v = \prod u_i^{v_i}$ for the same dimensional non-negative vectors u and v and we use here the convention $0^0 = 1$. The summation in (36) arises when multiple reactions in $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ are projected into the same reaction in $\mathcal{R}_L$.

9.2 Stationary distribution approximation under multiscaling model reduction

The main idea of the stationary distribution approximation shown in Section 2.4 in the main text is the multiscaling model reduction. In this section, we introduce the multiscaling for a stochastic reaction network system with reaction propensities of constant order.

Throughout this section we use the following notations. Let $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ be a network system with $\mathcal{S} = \{S_1, S_2, \ldots, S_{d+r}\}$ and let N be a scaling parameter. Let $X^N = (X_1^N, X_2^N, \ldots, X_{d+r}^N)$ be the associated scaled stochastic process with transition probabilities (12) such that each X_i^N represents the counts of species S_i. For a given initial condition $X^N(0)$, let

$$\mathcal{S}_L = \{S_i \in \mathcal{S} : X_i^N(0) = c_i\} \quad \text{and} \quad \mathcal{S}_H = \{S_i \in \mathcal{S} : X_i^N(0) = c_i N\}, \tag{37}$$

where c_i's are positive constants.

Then for a given collection of rate constants $\mathcal{K}$, we scale the system with the following reaction intensities.

$$\mathcal{K}^N = \left\{ \lambda_k^N = \frac{\kappa_{y \to y'}}{N^{\|q_H(y)\|_1}} \lambda_k : \lambda_k \in \mathcal{K} \right\}, \tag{38}$$

where $\|\cdot\|_1$ is the 1-norm.

For example, let $A + B \xrightarrow{0}$ be a reaction network system with $\mathcal{S}_L = \{A\}$ and $\mathcal{S}_H = \{B\}$. Let $x = (x_A, x_B)$ be a state of the associated stochastic process X^N with a scaling parameter N. Then the for a given reaction intensity $\lambda(x) = \kappa x_A x_B$ of the reaction $A + B \to 0$, we define a scaled reaction intensity $\lambda^N(x) = \frac{\kappa}{N^{\|q_H(A+B)\|_1}}\lambda(x) = \frac{\kappa}{N}x_A x_B$. Then the scaled stochastic process X^N has the transition probability

$$P(X^N(t + \Delta t) = x + (-1, -1)^T \mid X^N(t) = x) = \lambda^N(x)\Delta t + h(\Delta),$$

where h is defined as (12).

Having presented the above example, we now describe in more detail the procedure for a formal multiscaling model reduction. Note that in the example above, as long as $X_B(t)$ is of order N, the propensity is of order 1. Under this circumstance, we intuitively expect that X_B would not be substantially change because of the relatively low reaction propensity. Using this background, we can approximate the distribution of a stochastic system through multiscaling model reduction. The proof of the following theorem is provided in the separate paper [40].

Theorem 9.1. *Let $X^N = (X_1^N, X_2^N, \dots, X_{d+r}^N)$ be the stochastic processes associated with $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}^N)$ where $\mathcal{K}^N$ is as (38). For an initial condition $X(0)$, suppose $\mathcal{S} = \mathcal{S}_L \cup \mathcal{S}_H$ with $\mathcal{S}_L$ and $\mathcal{S}_H$ as in (37). Let X be the associated stochastic process for the projected network system $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L^N)$. Let further that $p_L^N(\cdot, t)$ and $p(\cdot, t)$ be the probability distributions for $q_L(X^N)$ and X at time t, respectively. Then for any $A \subset \mathbb{Z}_{\geq 0}^d$, we have*

$$|p^N(A, t) - p(A, t)| = O(N^\nu) \quad \text{for some } \nu \in (0, 1). \tag{39}$$

Remark 9.1. *In Theorem 9.1, if X admits a stationary distribution π, then*

$$|p^N(A, t) - \pi(A)| \leq |p^N(A, t) - p(A, t)| + |p(A, t) - \pi(A)|. \tag{40}$$

Hence, for fixed t, if $|p(A, t) - \pi(A)|$ is sufficiently small, then $p_L^N(A, t) \approx \pi(A)$ for large N.

For a special case, $p_L^N(A, t)$ could be explicitly estimated with a Poisson distribution π The following corollary follows by Theorem 4.2 and Theorem 9.1.

Corollary 9.2. *Under the same conditions in Theorem 9.1, suppose that $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L)$ has zero deficiency and is weakly reversible reaction network. Then for a positive steady state $c \in \mathcal{R}_{>0}^{|\mathcal{S}_L|}$ of the deterministic counter part (11) of $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L)$, we have*

$$\lim_{t\to\infty} \lim_{N\to\infty} p_L^N(x, t) = M \prod_{i=1}^{|\mathcal{S}_L|} \frac{c_i^{x_i}}{x_i!} \mathbb{1}_{\{x \in \mathbb{S}\}} \tag{41}$$

where $\mathbb{S}$ is the state space, and M is the normalizing constant.

9.3 Mean of the projected systems

In this section we consider the mean of the target species in a reduced system. As described in Theorem (4.2), when the reduced network $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L)$ has zero deficiency and is weakly reversible, the long-term behavior of the associated stochastic system follows a product form of Poissons (or

constrained Poissons). Moreover the mean of the system is determined by the positive steady state of the deterministic counterpart as described in Theorem (4.2). Hence, we control the mean of the target species approximately by using the positive steady state value of the reduced system. In fact, for a certain reduced network obtained from an ACR system, the steady state value of an ACR species is preserved in the reduced network.

Let π be a product form of Poisson distribution (or constrained Poissons) such as $\pi(x) = M\prod_{i=1}^{d}\frac{c_i^{x_i}}{x_i!}\mathbb{1}_{\{x\in\mathbb{S}\}}$ defined on a state space $\mathbb{S}$ with some normalizing constant M and some $c\in\mathbb{R}^d_{\geq 0}$. Let π_1 be the marginal distribution of the x_1-coordinate. If the support of π_1 is whole $\mathbb{Z}_{\geq 0}$ then π_1 is the Poisson distribution with rate c_1 because

$$\pi_1(x_1) = \sum_{x_2\in Z_{\geq 0}}\cdots\sum_{x_d\in Z_{\geq 0}}\pi(x) = \frac{1}{\sum_{x_i=0}^{\infty}\frac{c_1^{x_1}}{x_1!}}\frac{c_1^{x_1}}{x_1!} = e^{-c_1}\frac{c_1^{x_1}}{x_1!}$$

Thus the mean of the marginal distribution π_1 is equal to c_1. Note that the reactions in the basic ACR system (15) are always projected to $0 \underset{\theta}{\overset{\mu}{\rightleftharpoons}} X_1$, the state space of X_1 is always equal to $Z_{\geq 0}$ in the projected network system. Hence the mean of the target species in the controlled system is close to c_1, which is the positive steady state of the deterministic counter part.

Similarly to the stability analysis carried out in Section 6, we assume a given network system $(\mathcal{S},\mathcal{C},\mathcal{R},\mathcal{K})$ is ACR and admits conservation relations. With the same notation we used in Section 6, let

$$u^i\cdot x(0) = u^i\cdot x(t) = M_i \quad \text{for all } t \tag{42}$$

for some positive constants M_i's and for some vectors u^i's in $\mathbb{R}^d_{>0}$. Hence for each positive steady state x^* of the system, if the i-th entry of x^* corresponds to a non-ACR species, then x_i^* depends on the total concentrations M_i. In this case, we denote $x^*(M)$ a positive steady state on the stoichiometry class $S_{x(0)}$, where $M = (M_1, M_2, \ldots, M_k)$ such that $u^i\cdot x(0) = M_i$. We also denote the entry of $x^*(M)$ by $x_i^* = x_i^*(M)$. With these notations, the following theorem states that if a reduced network is obtained by freezing some non-ACR species of an ACR system, then the steady state value of an ACR species is preserved in the reduced system.

Theorem 9.3. *Let $(\mathcal{S},\mathcal{C},\mathcal{R},\mathcal{K})$ be a mass-action ACR system with $x'(t) = f(x(t))$. Suppose $(\mathcal{S},\mathcal{C},\mathcal{R},\mathcal{K})$ admits the conservation laws (42). We split $\mathcal{S} = \{S_1,\ldots,S_d,S_{d+1},\ldots,S_{d+r}\}$ into two disjoint subsets $\mathcal{S}_L = \{S_1,\ldots,S_d\}$ and $\mathcal{S}_H = \{S_{d+1},\ldots,S_{d+r}\}$, where none of the species in S_H is an ACR species. Let $\{x^*(M) : M = (M_1,M_2,\ldots,M_k)\in R^k_{>0}\}$ be the family of positive steady states of the system $(\mathcal{S},\mathcal{C},\mathcal{R},\mathcal{K})$. For the dynamics $x(t)$ of $(\mathcal{S},\mathcal{C},\mathcal{R},\mathcal{K})$, suppose there exist a $\widetilde{M} = (\widetilde{M_1},\ldots,\widetilde{M_k})$ such that*

$$x_{d+i}(0) = x^*_{d+i}(\widetilde{M_1},\ldots,\widetilde{M_k}) \quad \textit{for } i = 1,2,\ldots,r. \tag{43}$$

Then the projected system $(\mathcal{S}_L,\mathcal{C}_L,\mathcal{R}_L,\mathcal{K}_L)$ with the initial condition $q_L(x(0))$ has a positive steady state value at $\bar{x}^* = q_L(x^*(\widetilde{M}))$. In particular, if $S_i\in S_L$ is an ACR species with the ACR value x_i^*, then $\bar{x}_i^* = x_i^*$.

Proof. Let $\bar{x}'(t) = \bar{f}(\bar{x}(t))$ be the deterministic system of $(\mathcal{S}_L,\mathcal{C}_L,\mathcal{R}_L,\mathcal{K}_L)$. Note that $(\mathcal{S}_L,\mathcal{C}_L,\mathcal{R}_L)$ is obtained by freezing the species in $\mathcal{S}_H$ from $(\mathcal{S},\mathcal{C},\mathcal{R})$. Recall by the definition of $\mathcal{K}_L$ (38) that

each element $\mathcal{K}_L$ is a summation of some rate constants in $\mathcal{K}$ multiplied by the initial values of species in $\mathcal{S}_H$. Hence for any positive x_i's, we have

$$\bar{f}_i(x_1,\dots,x_d) = f_i(x_1,\dots,x_d,x_{d+1}(0),\dots,x_{d+r}(0)), \quad i=1,2,\dots,d.$$

For the $\widetilde{M}$, we have

$$\begin{aligned} 0 = f(x^*) &= f(x_1^*(\widetilde{M}),\dots,x_d^*,x_{d+1}^*(\widetilde{M}),\dots,x_{d+r}^*(\widetilde{M})) \\ &= f(x_1^*(\widetilde{M}),\dots,x_d^*(\widetilde{M}),x_{d+1}(0),\dots,x_{d+r}(0)) \\ &= \bar{f}(x_1^*(\widetilde{M}),\dots,x_d^*(\widetilde{M})). \end{aligned}$$

Hence $\bar{x}^* = (x_1^*(\widetilde{M}),\dots,x_d^*(\widetilde{M})) = q_L(x^*(\widetilde{M}))$ is a positive steady state of $(\mathcal{S}_L,\mathcal{C}_L,\mathcal{R}_L,\mathcal{K}_L)$.

Suppose $S_i \in S_L$ is an ACR species with the ACR value x_i^*. Since the ACR value is independent of any conservative quantities $M_1, M_2, \dots, M_k$ by definition, we have $x_i^*(\widetilde{M}) = x_i^*$. Consequently, $\bar{x}_i^* = x_i^*$. □

We demonstrate Theorem 9.3 with the following example.

Example 9.1. *Consider a network* $(\mathcal{S},\mathcal{C},\mathcal{R},\mathcal{K})$

$$A \underset{1}{\overset{1}{\rightleftharpoons}} B \qquad Z + A \xrightarrow{\theta} 2Z \qquad Z \xrightarrow{\mu} A$$

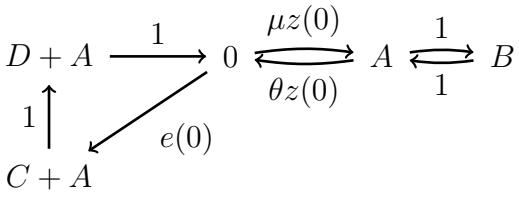
$$\begin{array}{ccc} C + A & \xrightarrow{1} & D + A \\ & {\scriptstyle 1}\nwarrow \quad \swarrow{\scriptstyle 1} & \\ & E & \end{array}$$

There are two conservation relations $a(0)+b(0)+z(0) = a(t)+b(t)+z(t) = M_1$ *and* $c(0)+d(0)+e(0) = c(t)+d(t)+e(t) = M_2$. *The mass action deterministic dynamics associated with* $(\mathcal{S},\mathcal{C},\mathcal{R},\mathcal{K})$ *is*

$$\begin{aligned} a'(t) &= -a(t) + b(t) - z(t)(\theta a(t) - \mu), \\ b'(t) &= a(t) - b(t), \\ z'(t) &= z(t)(\theta a(t) - \mu), \\ c'(t) &= -c(t)a(t) + e(t), \\ d'(t) &= -d(t)a(t) + c(t)a(t), \\ e'(t) &= -e(t) + d(t)a(t). \end{aligned}$$

Hence the positive steady state $x^* = (a^*,b^*,c^*,d^*,e^*,z^*) = (\frac{\mu}{\theta}, \frac{\mu}{\theta}, M_2\frac{\theta}{2\theta+\mu}, M_2\frac{\theta}{2\theta+\mu}, M_2\frac{\mu}{2\theta+\mu}, M_1 - 2\frac{\mu}{\theta})$. *This implies that species* A *and* B *are ACR species.*

We split $\mathcal{S}$ *into* $\mathcal{S}_L = \{A,B,C,D\}$ *and* $\mathcal{S}_H = \{E,Z\}$. *For a given initial condition* $x(0) = (a(0),b(0),c(0),d(0),e(0),z(0))$, *we have the folowing reduced network* $(\mathcal{S}_L,\mathcal{C}_L,\mathcal{R}_L,\mathcal{K}_L)$



With $M_1 = 2\frac{\mu}{\theta} + z(0)$ *and* $M_2 = \frac{2\theta+\mu}{\mu}e(0)$*, the condition* (43) *holds for species* E *and* Z*. Hence by Theorem* 9.3*, the positive steady state values of* A *and* B *in* $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L)$ *are same as the positive steady state values in* $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$*, which are* $\frac{\mu}{\theta}$ *for both species.* △

9.4 Approximation of controlled stochastic network with hybrid systems

In this section, we show that the basic ACR controller (15) can also be used to control a stochastic system under the classical scaling regime. To show that the target species in the scaled stochastic system follows approximately a Poisson distribution, we use a hybrid type system. This framework is introduced in [20].

We define $\gamma_k = 1$ if $q_H(y_k) > 0$ for a reaction $y_k \to y_k' \in \mathcal{R}$, otherwise $\gamma_k = 0$. As shown in Section 9.2, for a given set of intensity functions $\mathcal{K}$, we define new scaled reaction intensities as

$$\bar{\mathcal{K}}^N = \left\{ \bar{\lambda}_k^N = \frac{\kappa_{y\to y'}}{N^{\|q_H(y)\|_1 - \gamma_k}} \lambda_k : \lambda_k \in \mathcal{K} \right\}. \tag{44}$$

Suppose $\mathcal{S} = \mathcal{S}_L \cup \mathcal{S}_H$ as (37) for a reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$. Suppose further that the projected network $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L)$ has deficiency of zero and is weakly reversible. According to [20, Theorem 3.8 and Corollary 4.4], the system dynamics for $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \tilde{\mathcal{K}}^N)$ can be approximately studied with a hybrid system. The copy numbers of the species in $\mathcal{S}_L$ are modeled with a stochastic process, and the distribution of $q_L(X^N)$ is approximated with a product form of Poissons at a finite time t. On the other hand, the concentration of the species in $\mathcal{S}_H$ follows the deterministic dynamics. These two processes are coupled as the rate constants of the stochastic part are determined by the deterministic part, and the kinetics of the deterministic part also depends on the stationary mean of the stochastic part. We denote by $(\mathcal{S}_H, \mathcal{C}_H, \mathcal{R}_H, \bar{\mathcal{K}}_H)$ the deterministic part of the hybrid system. More precise definition of $(\mathcal{S}_H, \mathcal{C}_H, \mathcal{R}_H, \bar{\mathcal{K}}_H)$ can be found in [20].

In the scaled stochastic system modeled with $\bar{\mathcal{K}}^N$ defined at (44), let $m^N(t)$ be the mean copy number of the target species X_1 at time t such that $\lim_{N\to\infty} m^N(t) = m(t)$. That is, $m(t)$ approximates the mean of X_1 in the scaled stochastic system. The following Lemma provides the conditions guaranteeing that $m(t)$ converges in time to the desired value in a controlled system with the basic ACR controller. As we assumed for the basic ACR system in Section 5, we suppose that X_1 is in a given reaction network and Z is a newly introduced control species.

Lemma 9.4. *For a given reaction network, let* $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ *be the union of the given network and the basic ACR system* (15)*. Let* X^N *be the stochastic process associated with* $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \bar{\mathcal{K}}^N)$*, where* $\bar{\mathcal{K}}^N$ *is a set of scaled reaction intensities defined as* (44)*. For an initional state of* $X^N(0)$*, suppose* $X_1 \in \mathcal{S}_L$ *and* $Z \in \mathcal{S}_H$*. Suppose further that the conditions of Theorem 3.8 in [20] hold. If the concentration of* Z *in the deterministic part* $(\mathcal{S}_H, \mathcal{C}_H, \mathcal{R}_H, \bar{\mathcal{K}}_H)$ *converges to a positive steady state, as* $t \to \infty$*, then* $\lim_{t\to\infty} m(t) = \dfrac{\mu}{\theta}$.

Proof. Since Z is newly introduced species with the basic ACR system, Z is regulated with only two reactions $\emptyset \leftarrow Z \rightarrow 2A$ in $(\mathcal{S}_H, \mathcal{C}_H, \mathcal{R}_H, \bar{\mathcal{K}}_H)$. Let $z(t)$ be the concentration of species Z at time t. According to Theorem 3.8 in [20], the rate of reaction $\emptyset \leftarrow Z$ is $\mu z(t)$, and the rate of reaction $Z \rightarrow 2A$ is $\theta m(t) z(t)$. Then $z(t)$ solves a differential equation,

$$\frac{d}{dt} z(t) = z(t) \left(\theta m(t) - \mu\right),$$

Hence if $\lim_{t\to\infty} z(t) = z^*$ for some $z^* \in (0, \infty)$, then $\lim_{t\to\infty} (\theta m(t) - \mu) = 0$.

□

In Section 10.2, we use this lemma to show that the mean of the species X in the hybrid system in Figure (6)d converges to $\frac{\mu}{\theta}$.

9.5 Foster-Lyapunov criterion

By equation (39), it is important to show the term $|p(A,t) - \pi(A)|$ is small for the stationary distribution approximation (41) with a Poisson distribution. Basically the term $|p(A,t) - \pi(A)|$ tends to zero as $t \to \infty$ by the ergodic theorem [48]. In this section we introduce one of the most well-known theoretical frameworks, the so-called Foster-Lyapunov criterion [49] for the convergence of $|p^N(A,t) - \pi(A)|$ in t. The following theorem is a version of Theorem 6.1 in [49].

Theorem 9.5 (Foster-Lyapunov criterion for exponential ergodicity)**.** *Let X be a continuous-time Markov chain on a countable state space $\mathbb{S}$ with the infinitesimal generator $\mathcal{A}$ (14). Suppose there exists a positive function V on $\mathbb{S}$ satisfying the followings.*

1. *$V(x) \to \infty$ as $|x| \to \infty$, and*
2. *There are positive constants a and b such that*

$$\mathcal{A}V(x) \leq -aV(x) + b \quad \textit{for all} \quad x \in \mathbb{S}. \tag{45}$$

Then X admits a unique stationary distribution π. Furthermore, there exists positive constants η and C such that for any measurable set A and any state x,

$$|P(X(t) \in A | X(0) = x) - \pi(A)| \leq C(V(x) + 1)e^{-\eta t}.$$

For a finite time t, if the projected network system in Theorem 9.1 satisfies the conditions in Theorem 9.5, then the term $|p(A,t) - \pi(A)|$ in the right hand side of (40) can be small. Therefore $|p^N(A,t) - \pi(A)|$ in (40) can eventually be small enough with sufficiently large N.

10 Application of the stochastic dynamics

10.1 A receptor-ligand signaling model

In this section we provide the initial reaction propensities in the receptor-ligand signaling model described in Figure 4a. Note that we model the reaction propensities with stochastic mass-action

kinetics (13). Letting $N = 10^3$, we set the initial values $L(0) = 1.5N, P(0) = 100, R(0) = 2, Z(0) = N$, and we set zero initial values for the rest of species. The parameters are $\kappa_1^N = 1.24/(1.5N), \kappa_2^N = 1.37, \kappa_3^N = 1.41, \kappa_4^N = 1.79, \kappa_5^N = 1.02, \kappa_6^N = 1.36, \kappa_7^N = 1.97, \kappa_8^N = 1.11, \kappa_9^N = 1.55, \kappa_{10}^N = 1.01, \kappa_{11}^N = 1.34, \kappa_{12}^N = 0.5, \theta = 1/N$ and $\mu = 5/N$. Then for the initial condition $X(0) = (L(0), R_0(0), R(0), D(0), D_1(0), D_2(0), D_3(0), Z(0))$, we have the following propensities of reaction $L + R_0 \to R$, $R \to L + R_0$ and $2R \to D$.

$$\lambda_{L+R_0\to R}(X(0)) = \kappa_1 L(0)R_0(0) = 2.46, \quad \lambda_{R\to L+R_0}(X(0)) = \kappa_2 R(0) = 2.74$$
$$\lambda_{2R\to D}(X(0)) = \kappa_3 R(0)(R(0) - 1) = 2.82.$$

The propensities of all remaining reactions are zero since the initial counts of D, D_1, D_2 and D_3 are zero. As we mentioned in Section 2.4 of the main text, the initial propensities of each reaction is relatively small to the initial counts of species L and Z. Hence it needs a longer time to substantially deviate the counts of L and Z. Over a short term interval, each of L and Z in the associated stochastic process behaves as a constant function (Figure 4c). Hence the reduced model obtained by freezing L and Z at their initial values approximates the original controlled system.

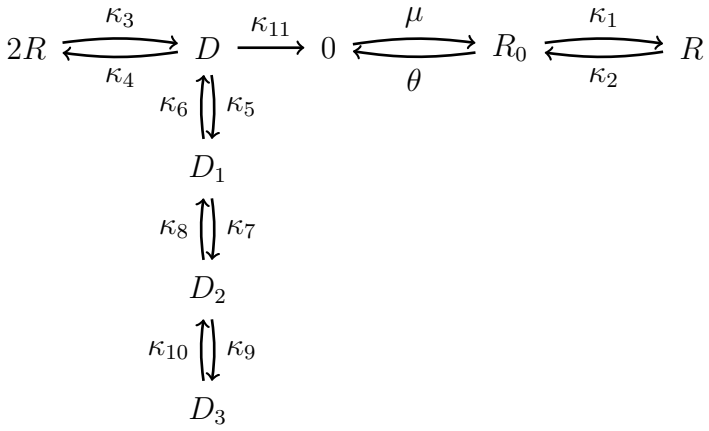
By using Theorem 9.1, we show this approximation more precisely. We let $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ be the controlled system in Figure 4 a. We choose the initial values and the set $\mathcal{K}^N$ of parameters as introduced above with the scaling parameter N. Note that $\mathcal{K}^N$ satisfies (38). We split $\mathcal{S}$ into $\mathcal{S}_L = \{R_0, R, D, D_1, D_2, D_3\}$ and $\mathcal{S}_H = \{L, Z\}$. Then the reduced network $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L)$ is the network in Figure 4 b. The deficiency of the reduced network is 0 as the number of complexes is 8, there are two linkage classes, and the rank of the stoichiometry matrix is 6. The reduced network is also weakly reversible because each linkage class is strongly connected. Hence Corollary 9.2 implies the distribution p_L^N of S_L species at $t = 150$ in $(\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K})$ is estimated by a product form of Poissons as described at (41).

In order to approximate the mean of R_0 in the controlled system $(\mathcal{S}, \mathcal{C}, \mathcal{R})$, we show that the positive steady state value of R_0 in $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L)$ is $\frac{\mu}{\theta} = 5$. Theorem 9.3 can be used to show that the mean, but using deficiency zero condition of the reduced network provides a much simpler way. Since $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L)$ has zero deficiency and is weakly reversible, the associated mass action deterministic dynamics is *complex balanced* [16, 17]. This means that for each complex, all 'in-flows' and 'out-flows' are balanced at each positive steady state. Hence for the zero complex in $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L)$, the in-flow is θr_0^* and the out-flow is μ for the positive steady value r_0^* of R_0. Therefore they are balanced at $r_0^* = \frac{\mu}{\theta}$.

We now investigate the accuracy of this approximation. Let $p_L^N(\cdot, t)$ and $p(\cdot, t)$ denote the distribution of species S_L in the controlled receptor-ligand system and the distribution of the reduced system, respectively. We further let π be the product form of Poissons stationary distribution of the reduced system. As shown in (40), for a small error between $p_L^N(\cdot, t_0)$ and π with a fixed time $t_0 = 150$, we need a fast convergence for $p(\cdot, t)$ to π.

With a slight modification on the reduced network, we show that how to use the Foster-Lyapunov criterion in Theorem 9.5 to show the convergence rate of $p(\cdot, t)$ to π in time t. In order to construct a Lyapunov function explicitly, we add a degradation of D to the reduced model in

Figure 4b. Hence let $(\mathcal{S}_L, \mathcal{C}_L, \mathcal{R}_L, \mathcal{K}_L)$ be a system described with the following reaction network.



(46)

Let $x = (x_1, x_2, x_3, x_4, x_5, x_6)$ be a vector each of whose entries represents the copy numbers of R_0, R, D, D_1, D_2 and D_3, respectively. We use a linear Lyapunov function $V(x) = \sum_{i=1}^{6} v_i x_i$ with some positive vector $v = (v_1, v_2, \ldots, v_d)$. The work in [50] exploited details about linear Lyapunov functions for stochastic reaction networks. By the definition of the generator $\mathcal{A}$ (14), we have

$$\begin{aligned}\mathcal{A}V(x) =&\kappa_{10}(v_5 - v_6)x_6 + (\kappa_9(v_6 - v_5) + \kappa_8(v_4 - v_5))x_5 + (\kappa_7(v_5 - v_4) + \kappa_6(v_3 - v_4))x_4 \\ &+ (\kappa_5(v_4 - v_3) + \kappa_4(2v_2 - v_3) - \kappa_{11}v_3)x_3 + +\kappa_2(v_1 - v_2)x_2 \\ &+ (\kappa_1(v_2 - v_1) - \theta v_1)x_1 + (\kappa_3(v_3 - 2v_2))x_2^2 + \mu.\end{aligned} \tag{47}$$

Let $h_i = v_{i+1} - v_i$ for $i = 1, 3, 4, 5$ and $h_2 = v_3 - 2v_2$. We will choose h_i's with which all the coefficients on the right hand side of (47) are strictly negative numbers. Hence, we choose h_i's such that

$$\begin{aligned}&h_1 < 0, \quad h_2 > 0, \quad \frac{\kappa_5}{\kappa_{11}}h_3 - \frac{\kappa_4}{\kappa_{11}}h_2 < v_3 = 2v_1 + 2h_1 + h_2 \\ &\frac{\kappa_7}{\kappa_6}h_4 < h_3, \quad \frac{\kappa_9}{\kappa_8}h_5 < h_4, \quad \text{and} \quad h_5 > 0.\end{aligned} \tag{48}$$

With a sufficiently large v_1, we can find h_i's satisfying (48). Then we have

$$\mathcal{A}V(x) = -c_1x_1 - c_2'x^2 + c_2x_2 - c_3x_3 - c_4x_4 - c_5x_4 - c_6x_6,$$

for some $c_i > 0$ for $i = 1, 2, \ldots, 6$ and $c_2' > 0$. Hence (45) holds, and for the distribution $p(\cdot, t)$ of (46) and its stationary distribution π, we have the exponential decay of $|p(A, t) - \pi(A)|$ for any $A \subset Z_{\geq 0}^6$, as $t \to \infty$.

10.2 A Dimer-Catalyzer Model

Recall that we used the hybrid system shown in Figure 6d to approximate the distribution of X in the controlled dimer-catalyzer system that we introduced in Figure 6a. In this section, by using Lemma 9.4 we prove that $m(t)$, the mean of X at time t in the hybrid system, converges to $\frac{\mu}{\theta}$ under a mild condition, as $t \to \infty$.

Let $x_1(t), c(t), c_p(t), c_{pp}(t), x^*(t)$ and $z(t)$ be the solution to the deterministic part of the hybrid system shown in Figure 6d. Let further $m(t) = \dfrac{\kappa_1 x^*(t) + \mu z(t)}{\kappa_2 c_{pp}(t) + \theta z(t)}$ and $\ell(t) = \dfrac{\kappa_4 c(t)}{\kappa_3 x_1(t)}$ be the mean of X and X_2 at time t, respectively. Then the deterministic system is governed by the following system of ordinary differential equations,

$$\begin{aligned}
&\frac{d}{dt}x_1(t) = \kappa_4 c(t) - \kappa_3 x_1(t)\ell(t) = 0,\\
&\frac{d}{dt}c(t) = \kappa_3 x_1(t)\ell(t) + \kappa_6 c_p(t) - (\kappa_4 + \kappa_5)c(t) = \kappa_6 c_p(t) - \kappa_5 c(t),\\
&\frac{d}{dt}c_p(t) = \kappa_5 c(t) + \kappa_8 c_{pp}(t) - (\kappa_6 + \kappa_7)c_p(t),\\
&\frac{d}{dt}c_{pp}(t) = \kappa_7 c_p(t) - \kappa_8 c_{pp}(t),\\
&\frac{d}{dt}x^*(t) = \kappa_2 c_{pp}(t)m(t) - \kappa_1 x^*(t), \quad \text{and}\\
&\frac{d}{dt}z(t) = z(t)(\theta m(t) - \mu).
\end{aligned} \tag{49}$$

Let $x_1(0), c(0), c_p(0), c_{pp}(0), x^*(0)$ and $z(0)$ be the initial values of the system (49). Note that $\dfrac{d}{dt}x^*(t) + \dfrac{d}{dt}z(t) = 0$ and $\dfrac{d}{dt}c(t) + \frac{d}{dt}c_p(t) + \dfrac{d}{dt}c_{pp}(t) = 0$ for all t. We let $M = x^*(0) + z(0) = x_1(t) + z(t)$ and $L = c(0) + c_p(t) = c(t) + c_p(t) + c_{pp}(t)$ be the conserved quantities.

By using the second, third and fourth equations in (49), there is a single positive steady state,

$$(\bar{c}, \bar{c}_p, \bar{c}_{pp}) = \left(\frac{\kappa_6\kappa_8 L}{\kappa_6\kappa_8 + \kappa_5\kappa_8 + \kappa_5\kappa_7}, \frac{\kappa_5\kappa_6\kappa_8 L}{\kappa_6^2\kappa_8 + \kappa_5\kappa_6\kappa_8 + \kappa_5\kappa_6\kappa_7}, \frac{\kappa_5\kappa_6\kappa_7\kappa_8 L}{\kappa_6^2\kappa_8^2 + \kappa_5\kappa_6\kappa_8^2 + \kappa_5\kappa_6\kappa_7\kappa_8}\right),$$

for $x_1(t), c(t), c_p(t)$ and $c_{pp}(t)$. In order to study the stability of this positive steady state, we consider the following linear system for c, c_p and c_{pp},

$$\frac{d}{dt}\begin{pmatrix} c \\ c_p \\ c_{pp} \end{pmatrix} = \begin{pmatrix} -\kappa_5 & \kappa_6 & 0 \\ \kappa_5 & -\kappa_6 - \kappa_7 & \kappa_8 \\ 0 & \kappa_7 & -\kappa_8 \end{pmatrix}\begin{pmatrix} c \\ c_p \\ c_{pp} \end{pmatrix}.$$

The eigenvalues of the matrix above is

$$\begin{aligned}
&\lambda_1 = 0,\\
&\lambda_2 = -\frac{1}{2}(\kappa_5 + \kappa_6 + \kappa_7 + \kappa_8) - \frac{1}{2}\sqrt{(\kappa_5 + \kappa_6 - \kappa_7 - \kappa_8)^2 + 4\kappa_6\kappa_7},\\
&\lambda_2 = -\frac{1}{2}(\kappa_5 + \kappa_6 + \kappa_7 + \kappa_8) + \frac{1}{2}\sqrt{(\kappa_5 + \kappa_6 - \kappa_7 - \kappa_8)^2 + 4\kappa_6\kappa_7}.
\end{aligned}$$

Since $(\kappa_5 + \kappa_6 + \kappa_7 + \kappa_8)^2 > (\kappa_5 + \kappa_6 - \kappa_7 - \kappa_8)^2 + 4\kappa_6\kappa_7$, the eigenvalues λ_2 and λ_3 are strictly negative. Thus

$$\begin{pmatrix} c(t) \\ c_p(t) \\ c_{pp}(t) \end{pmatrix} = v_1 + v_2 e^{-\lambda_2 t} + v_3 e^{-\lambda_3 t},$$

where v_i's are the corresponding eigenvectors. In particular, $v_1 = (\bar{c}, \bar{c}_p, \bar{c}_{pp})$. This implies that $\lim_{t\to\infty} c_{pp}(t) = \bar{c}_{pp}$.

Now in the following proposition we introduce a sufficient condition for $z(t)$ to converge to some positive steady state.

Proposition 10.1. *Suppose that $\theta\kappa_1 M > \mu\kappa_2\bar{c}_{pp}$. Then $z(t)$ in (49) converges to $\dfrac{\theta\kappa_1 M - \mu\kappa_2\bar{c}_{pp}}{\theta\kappa_1}$, as $t \to \infty$.*

Proof. By using the conservative quantity $M = x^*(t) + z(t)$, the differential equation for z in (49) can be written as

$$\frac{d}{dt}z(t) = z(t)\left(\frac{\theta\kappa_1 M - \mu\kappa_2 c_{pp}(t) - \theta\kappa_1 z(t)}{\kappa_2 c_{pp}(t) + \theta z(t)}\right) = \frac{\theta\kappa_1 z(t)}{\kappa_2 c_{pp}(t) + \theta z(t)}(\alpha(t) - z(t)),$$

where $\alpha(t) = M - \dfrac{\mu\kappa_2}{\theta\kappa_1}c_{pp}(t)$.

Let $\bar{\alpha} = M - \dfrac{\mu\kappa_2}{\theta\kappa_1}\bar{c}_{pp}$. Let also $\epsilon > 0$ be an arbitrarily small number.Since $\lim_{t\to\infty} c_{pp}(t) = \bar{c}_{pp}$, there exists a $T > 0$ such that $|\alpha(t) - \bar{\alpha}| < \epsilon$ when $t > T$. We denote $R_{-\epsilon} = \bar{\alpha} - 2\epsilon$ and $R_{+\epsilon} = \bar{\alpha} + 2\epsilon$. Then clearly $\alpha(t) \in [R_{-\epsilon}, R_{+\epsilon}]$ for all $t > T$.

In the rest of the proof, we suppose $t > T$. Note that if $z(t) < R_{-\epsilon}$, then

$$\frac{d}{dt}z(t) = \frac{\theta\kappa_1 z(t)}{\kappa_2 c_{pp}(t) + \theta z(t)}(\alpha(t) - z(t)) > \frac{\theta\kappa_1 z(t)}{\kappa_2 c_{pp}(t) + \theta z(t)}(\alpha(t) - \bar{\alpha} + 2\epsilon) > \frac{\theta\kappa_1 z(t)}{\kappa_2 L + \theta M}\epsilon$$

Note further that if $z(t) > R_{+\epsilon}$, then

$$\frac{d}{dt}z(t) = \frac{\theta\kappa_1 z(t)}{\kappa_2 c_{pp}(t) + \theta z(t)}(\alpha(t) - z(t)) < \frac{\theta\kappa_1 z(t)}{\kappa_2 c_{pp}(t) + \theta z(t)}(\alpha(t) - \bar{\alpha} - 2\epsilon) < -\frac{\theta\kappa_1 z(t)}{\kappa_2 L + \theta M}\epsilon$$

Therefore there exists $T_1 = \inf\{t \geq T : z(t) \in [R_{-\epsilon}, R_{+\epsilon}]\} < \infty$. Let $T_2 = \inf\{t > T_1 : z(t) = \bar{\alpha}\}$. We first consider the case $z(T_1) = R_{-\epsilon}$ and $T_2 < \infty$. If there exists $t > T_2$ such that $z(t) > \alpha(t)$, then by using the continuity of $z(t)$,

$$z(t) = z(T_2) + \int_{T_2}^{t}\frac{d}{ds}z(s)ds = \alpha(t) + \int_{T_2}^{t}\frac{\theta\kappa_1 z(s)}{\kappa_2 c_{pp}(s) + \theta z(s)}(\alpha(s) - z(s))ds < \alpha(t).$$

Hence it is contradiction to $z(t) > \alpha(t)$. Thus $R_{-epsilon} < z(t) < \alpha(t)$ for all $t > T_2$. Now we consider the case $z(T_1) = R_{-\epsilon}$ and $T_2 = \infty$. Then for any $t > T_1$, it follows that

$$z(t) = z(T_1) + \int_{T_1}^{t}\frac{d}{ds}z(s)ds = R_{-epsilon} + \int_{T_2}^{t}\frac{\theta\kappa_1 z(s)}{\kappa_2 c_{pp}(s) + \theta z(s)}(\alpha(s) - z(s))ds > R_{-\epsilon}$$

as $z(t) < \alpha(t)$ for any $t > T_1$. Hence $R_{-\epsilon} < z(t) < \alpha(t)$ for all $t > T_1$. Hence we conclude that when $z(T_1) = R_{-\epsilon}$, there exists $T_1' \geq T_1 \geq T$ such that such that $\alpha(t) < z(t) < R_{+\epsilon}$ for all $t > T_1'$. In the same way, it follows that when $z(T_1) = R_{+\epsilon}$ there exists $T_2' \geq T_1 \geq T$ such that $\alpha(t) < z(t) < R_{+\epsilon}$ for all $t > T_3$.

Consequently, there exists $T' \geq T$ such that $z(t) \in [R_{-\epsilon}, R_{+\epsilon}]$ for all $t > T'$. Since ϵ can be chosen arbitrarily small with sufficiently large T, $z(t)$ converges to $\bar{\alpha}$, as $t \to \infty$. Obviously $\bar{\alpha}$ is positive by the assumption $\theta\kappa_1 M > \mu\kappa_2\bar{c}_{pp}$.

□

Proposition 10.1 shows that for any choice of system parameters κ_i, if either the initial value of x^* or z is large enough, then $z(t)$ converges to a positive steady state, as t goes to ∞. Hence by using Lemma 9.4, we conclude that $\lim_{t\to\infty} m(t) = \frac{\mu}{\theta}$ if we input sufficiently large initial concentration of the control species Z.

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