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Scale–invariance in singularly perturbed systems

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***Abstract*— The property termed scale-invariance, or fold-change detection, represents a phenomenon that is observed in a variety of biological systems, ranging from bacterial to eukaryotic signaling pathways. Mathematically, it represents invariance of the complete output trajectory with respect to a rescaling of input magnitudes. In the systems biology literature, an often-discussed motif for approximate fold-change detection is based on a time-scale separation in which output variables respond faster than internal components do. This paper shows that there is a lower bound on the scaling error for systems based on this property, independently of the magnitude of the time-scale separation. Furthermore, the paper discusses how adaptation and scale invariance properties often fail to hold when the effect of molecular noise is taken into account.**

I. INTRODUCTION

In biological systems, the problem of asymptotic disturbance rejection to constant inputs is often called "(perfect) adaptation" [1]. Here we deal with a stronger property, namely scale-invariance (SI) of responses. Let us consider two step inputs u_1 and u_2 which are scaled versions of each other: $u_2(t) = pu_1(t)$, for some positive number or "scale" p. Adaptation means that, no matter which input we employ, the output will asymptotically converge to the same value, while scale-invariance means that the entire transient response will be the same; see the journal paper [7] for more motivational details and references to the biology literature.

One of two main biomolecular mechanisms (the other is integral feedback) that has been discussed as providing scale-invariance is the *IFFL1 (incoherent feedforward loop)* motif. In such a system, the input u activates an internal state coordinate x which then activates a second state coordinate y, seen as the output of the system, but the input also directly represses the output, see Figure 1(a). However, the cartoon diagram shown in Figure 1(a) can be instantiated in alternative molecular realizations, which might significantly differ in their dynamic response, see for example Figures 1(b) and (c). It turns out that the first of these has the SI property, but not the second one.

Indeed, let $(x(t), y(t))$ be a solution corresponding to an input $u(t)$, for the system described by Figure 1(b) when modeled as follows:

$$\begin{aligned} \dot{x} &= \alpha u - \delta x, \\ \dot{y} &= \beta \tfrac{x}{u} - \gamma y\,. \end{aligned} \tag{1}$$

Then, $(px(t), y(t))$ is a solution corresponding to the input

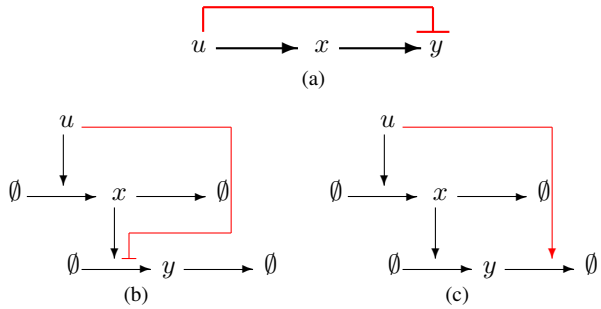

Fig. 1: Incoherent feedforward motif type 1 (IFFL1) (a) and its two realizations: input (b) inhibits output formation; or (c) enhances output degradation.

$pu(t)$:

$$\begin{aligned} \dot{(px)} &= \alpha(pu) - \delta(px), \\ \dot{y} &= \beta \frac{px}{pu} - \gamma y\,. \end{aligned}$$

In particular, for example, if we consider a step input that jumps at time $t = 0$ and an initial state at time $t = 0$ that has been pre-adapted to the input u for $t < 0$ (i.e., $x(0) = \alpha u_0/\delta$, where u_0 is the value of u for $t < 0$), then the solution is the same as when applying input pu for $t > 0$, but starting from the respective pre-adapted state $(p\alpha u_0/\delta)$. On the other hand, the SI property *fails* for the system shown in Figure 1(c), and modeled by:

$$\begin{aligned} \dot{x} &= \alpha u - \delta x, \\ \dot{y} &= \beta x - \gamma u y, \end{aligned} \tag{2}$$

because the scaling $x \mapsto px$ and $u \mapsto pu$ does not leave the y equation invariant, and one can prove that no possible equivariant group action on states is compatible with output invariance, as discussed in [6], where a systematic analysis of the SI property was carried out.

A. Approximate scale-invariance via time-scale separation

Systems as in Figure 1(c) and modeled by (2) satisfy an *approximate* SI property, provided that the parameters β and γ are large enough so that a time-scale separation property holds. In fact, we showed in [8], that every three node enzymatic network of a certain type that is approximately SI must necessarily rely upon this mechanism of time scale separation, which shows the interest of this class of systems. Redefining parameters (see [7] for details) and writing our system in singular perturbation form:

$$\begin{aligned} \dot{x} &= \alpha u - \delta x, \\ \varepsilon \dot{y} &= \beta x - \gamma u y\,, \end{aligned} \tag{3}$$

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where we think of $0 < \varepsilon \ll 1$ as a small parameter but the remaining parameters are all $O(1)$. The reduced system at a slow time-scale, obtained by replacing $y(t)$ by its equilibrium value (set $\varepsilon = 0$) is described by the one-dimensional system

$$\dot{x} = \alpha u - \delta x\,,$$

with output

$$y(t) \approx \frac{\beta x(t)}{\gamma u(t)}\,, \tag{4}$$

and it is now clear that scaling $u \mapsto pu$ and $x \mapsto px$ results in (approximately) the same output.

Intuitively, since the approximation in (4) increases in accuracy as $\varepsilon \to 0$, one would expect that the scale-invariance error should become negligible as $\varepsilon \to 0$. Perhaps surprisingly, this intuition turns out to be wrong, as we discuss next.

B. A fundamental limitation

Our main results show that, no matter how small ε is, there is always a minimal possible SI error, meaning a minimal positive difference in instantaneous values of the output $y_1(t)$ and the output $y_p(t)$ for, respectively, inputs $u(t)$ and $pu(t)$. See Figure 2 for an example. In Section II, we summarize

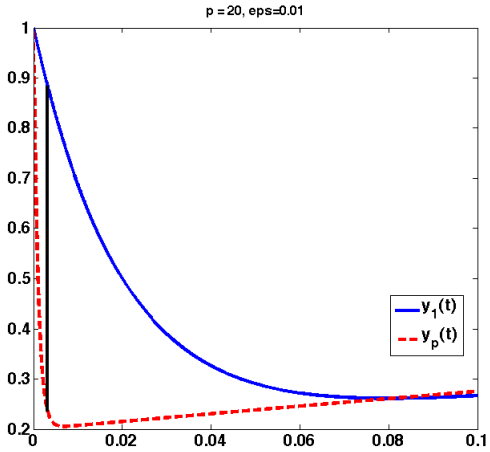

Fig. 2: Output of the system in Figure 1(c) and (3). All parameters except ε are 1. Original (blue) and p-scaled (red) responses are shown. Pre-adaptation value of input is $u_0 = 0.1$, stepping to $u^* = 0.5$ at $t = 0$. Here $\varepsilon = 0.01$ and $p = 20$. The maximal magnitude of the SI error is depicted by a black segment

our main theorem on fundamental limitations, referring to the journal paper [7] for details on that part. In Section III, we briefly discuss the use of the boundary function method in singular perturbations to obtain estimates of the minimal SI error, and in Section IV we make remarks on the limitations involved by introducing stochasticity.

II. Lower bounds on scale-invariance error

A. A simple feedforward system

We start by considering the response of the singularly perturbed IFFL described in (3) to a step input $u(t)$ which switches from the value $u(t) = u_0$ for $t < 0$ to a different value $u(t) = u^*$ for $t > 0$, under the assumption that the states x and y had converged to a steady state by $t = 0$, and the response to the input $pu(t)$. The response for $t > 0$ will be given by the solution of the ODE with initial condition $x(0) = \frac{\alpha}{\delta}u_0$ and $y(0) = \frac{\alpha\beta}{\delta\gamma}$, and input $u(t) \equiv u^*$ for $t > 0$. In the p-scaled case, the initial state will be $x(0) = \frac{\alpha}{\delta}pu_0$, and the same $y(0)$, now using the input $u(t) \equiv pu^*$ for $t > 0$. A nondimensionalization analysis (see [7] for details) allows us to take, without loss of generality, $\alpha = \beta = \delta = \gamma = 1$ in our subsequent analysis. We use $\|y - w\|_{[0,T]}$ to indicate the maximum possible value of the difference $|y(t) - w(t)|$ between two functions defined on an interval $t \in [0, T]$. The main result for this example is as follows.

Proposition 2.1: Consider solutions $(x_i^\varepsilon(t), y_i^\varepsilon(t))$ of the following two initial value problems:

$$\begin{array}{rclcl} \dot{x}_1^\varepsilon & = & u^* - x_1^\varepsilon, & & x_1^\varepsilon(0) \;=\; u_0\,, \\ \varepsilon\dot{y}_1^\varepsilon & = & x_1^\varepsilon - u^* y_1^\varepsilon, & & y_1^\varepsilon(0) \;=\; 1 \\ \dot{x}_2^\varepsilon & = & u^* - x_2^\varepsilon, & & x_2^\varepsilon(0) \;=\; pu_0 \\ \varepsilon\dot{y}_2^\varepsilon & = & x_2^\varepsilon - pu^* y_2^\varepsilon, & & y_2^\varepsilon(0) \;=\; 1 \end{array} \tag{5}$$

where ε, u^*, u_0, and p are nonzero positive numbers, and we assume that $p \neq 1$, $u_0 \neq u^*$. Define $M = M(u^*, u_0, p) > 0$ by:

$$M \;:=\; \left|1 - \frac{u_0}{u^*}\right| p^{\frac{p}{1-p}}\,|1 - p|\;. \tag{6}$$

Then, for any $0 < M' < M < M''$, there exist two numbers $\varepsilon_0 = \varepsilon_0(u^*, u_0, 1, p, M', M'')$, and $\delta = \delta(u^*, u_0, p, M', M'') > 0$, such that:

$$M' \;\leq\; \|y_1^\varepsilon - y_2^\varepsilon\|_{[0,\delta]} \;\leq\; M'' \qquad \forall\, 0 < \varepsilon \leq \varepsilon_0\,. \tag{7}$$

Since M' and M'' can be taken arbitrarily close to M, this result tells us, in particular, that $\|y_1^\varepsilon - y_2^\varepsilon\|_{[0,\delta]} \approx M$ for all $0 < \varepsilon \ll 1$, and δ small. In other words, the positive number given in formula (6), which does not depend on ε, provides a fundamentally irreducible error as $\varepsilon \to 0$, for any nontrivial scaling ($p \neq 1$) and any nontrivial step input ($u_0 \neq u^*$). A proof is provided in the journal version of this paper, see [7].

B. The general case

We also provide an abstract comparison theorem that generalizes Proposition 2.1 to arbitrary systems. The theorem is formulated and proved (see [7]) for two arbitrary singularly-perturbed non-autonomous initial-value problems (IVPs), as follows:

$$(S_1)\;\begin{cases} \dot{x}_1 \;=\; f_1(x_1, y_1, t),\; x_1(0) = \xi_1, \\ \varepsilon\dot{y}_1 \;=\; g_1(x_1, y_1, t),\; y_1(0) = \kappa_1, \end{cases} \tag{8}$$

$$(S_2)\;\begin{cases} \dot{x}_2 \;= f_2(x_2, y_2, t),\; x_2(0) = \xi_2, \\ \varepsilon\dot{y}_2 = g_2(x_2, y_2, t),\; y_2(0) = \kappa_2. \end{cases}$$

Here, (x_i, y_i), $(\xi_i, \kappa_i) \in X \times Y$, where X and Y are open sets, $X \subseteq \mathbb{R}^n$ and $Y \subseteq \mathbb{R}^s$. The functions f_i and g_i are of

class C^1 with respect to x, y, and t, $i = 1, 2$. Consider also the following two "reduced" ODE systems:

$$\begin{aligned} &(A_1)\ Y_1' = g_1(\xi_1, Y_1, 0),\ Y_1(0) = \kappa_1, \\ &(A_2)\ Y_2' = g_2(\xi_2, Y_2, 0),\ Y_2(0) = \kappa_2, \end{aligned} \tag{9}$$

obtained when ε is ignored but x_1 and x_2 are replaced by their initial values ξ_2 and ξ_1 in S_1 and S_2, respectively. (Here we use primes $'$ to indicate time derivatives, to reflect the fact that this system is obtained through a rescaling of time, as discussed later.)

To apply the result that follows to the SI problem, suppose that we are given a system of the generic form

$$\begin{aligned} \dot{x} &= f(x, y, u) \\ \varepsilon\dot{y} &= g(x, y, u) \end{aligned}$$

where the input as well as the state vector (x, y) are of arbitrary dimensions. We think of the components of y as an output, and want to compare the outputs associated to two inputs $u(t)$ and $pu(t)$, for $t > 0$, when initial states might themselves depend on the values of $u(t)$ and $pu(t)$ for $t < 0$. This latter dependence is encapsulated in the initial states (ξ_1, κ_1) and (ξ_2, κ_2) respectively, to the systems (S_1) and (S_2) obtained by looking at the original system and its version under input pu.

Since the transient SI-error occurs within a thin boundary layer adjacent to the perturbation moment $t = 0$, as can be seen in Figure 2, we analyze nonlinear effects occurring within small time intervals, by using the stretched time $\tau = t/\varepsilon$. Substituting $t = \varepsilon\tau$ into (8), we obtain

$$(R_1)\begin{cases} X_1' &= \varepsilon f_1(X_1, Y_1, \varepsilon\tau),\ X_1(0) = \xi_1, \\ Y_1' &= g_1(X_1, Y_1, \varepsilon\tau), \quad Y_1(0) = \kappa_1, \end{cases} \tag{10}$$

$$(R_2)\begin{cases} X_2' &= \varepsilon f(X_2, Y_2, \varepsilon\tau),\ X(0) = \xi_2, \\ Y_2' &= g(X_2, Y_2, \varepsilon\tau), \quad Y(0) = \kappa_2, \end{cases} \tag{11}$$

where $(\cdot)' = d(\cdot)/d\tau$, and all functions are continuously-differentiable with respect to the variables, the initial conditions and the parameter $\varepsilon > 0$ as discussed above.

In contrast to the *singularly*-perturbed systems (S_1) and (S_2), both systems (R_1) and (R_2) are *regularly*-perturbed with respect to ε. It follows that the SI-error should be already detected at $\varepsilon = 0$ in which case the systems (R_1) and (R_2) can be further reduced to the following associated systems shown in (9). We will denote the solutions of the systems (R_i) by $X_i^\varepsilon(\tau)$ and $Y_i^\varepsilon(\tau)$, $i = 1, 2$.

Theorem 1: Assume that the solution $(x_i^\varepsilon(t), y_i^\varepsilon(t))$ of the system (S_i) is defined on $[0, \infty)$ for all $\varepsilon \in (0, \varepsilon_0]$ with some $\varepsilon_0 > 0$, $i = 1, 2$. Let $Y_i^0(\cdot)$ be the solution of the associated system (A_i), $i = 1, 2$. Then, for each $\varepsilon \in (0, \varepsilon_0]$ and each $0 \le \tau_0 < \infty$, we have:

$$M_{\tau_0} - \varepsilon N_{\tau_0} \le \|y_1^\varepsilon - y_2^\varepsilon\|_{[0,\varepsilon\tau_0]} \le M_{\tau_0} + \varepsilon N_{\tau_0}, \tag{12}$$

where M_{τ_0} and N_{τ_0} are defined as follows:

$$\begin{aligned} M_{\tau_0} &= \left|Y_2^0(\tau_0) - Y_1^0(\tau_0)\right|, \\ N_{\tau_0} &= \max_{0\le\varepsilon\le\varepsilon_0} \left\| \frac{\partial Y_1^\varepsilon(\cdot)}{\partial\varepsilon} \right\|_{[0,\tau_0]} + \max_{0\le\varepsilon\le\varepsilon_0} \left\| \frac{\partial Y_2^\varepsilon(\cdot)}{\partial\varepsilon} \right\|_{[0,\tau_0]}. \end{aligned} \tag{13}$$

Theorem 1 implies that if the solutions of the associated IVP (9) are different, that is, if $Y_1^0(\tau) \neq Y_2^0(\tau)$ for some τ, then as $\varepsilon \to 0$ there will always exist a minimal possible nonzero difference (in supremum norm) between the solutions of the corresponding singularly-perturbed problems (S_1) and (S_2), approximately equal to M_{τ_0}. The effect is solely determined by the properties of the fast subsystem. A proof is given in the journal paper [7].

III. Asymptotic expansions

The main result does not provide explicit error estimates. We next sketch briefly how one may obtain estimates through the use of tools from singular perturbation theory. Consider solutions $(x(t;\varepsilon), y(t;\varepsilon))$ and $(z(t;\varepsilon,p), w(t;\varepsilon,p))$ of the following two initial value problems:

$$\begin{aligned} \dot{x} &= f(x, y, u(t)), \quad x(0) = \sigma_1(u_0), \\ \varepsilon\dot{y} &= g(x, y, u(t)), \quad y(0) = \sigma_2(u_0), \end{aligned} \tag{14}$$

$$\begin{aligned} \dot{z} &= f(z, w, pu(t)), \quad z(0) = \sigma_1(pu_0), \\ \varepsilon\dot{w} &= g(z, w, pu(t)), \quad w(0) = \sigma_2(pu_0). \end{aligned} \tag{15}$$

where the assumptions are the same as before, and $\sigma_1(u_0)$ and $\sigma_2(u_0)$ are the pre-adapted steady states for x and y, when an input u_0 has been applied. Similarly, for z and w.

As before, our goal is to investigate the behavior of the scale-invariance error function $E(t;\varepsilon,p)$ defined as:

$$E(t;\varepsilon,p) = w(t;\varepsilon,p) - y(t;\varepsilon) \tag{16}$$

on t, p, and ε as $\varepsilon \to 0+$.

As in [6], we study the class of systems which satisfy the following homogeneity properties:

$$\begin{aligned} &\sigma(pu) = p\sigma(u), \\ &f(px, y, pu) = pf(x, y, u), \\ &g(px, y, pu) = pg(x, y, u). \end{aligned} \tag{17}$$

Then (15) can be rewritten in the form:

$$\begin{aligned} \dot{z} &= f(z, w, pu(t)), \quad z(0) = p\sigma_1(u_0), \\ \varepsilon\dot{w} &= g(z, w, pu(t)), \quad w(0) = \sigma_2(u_0). \end{aligned} \tag{18}$$

To estimate a lower bound for the SI-error in cases where an analytical solution of the system of ODEs cannot be found, it is convenient to employ the theory of singular perturbations [5], [12], [3], and in particular, make use of the *method of boundary functions* [12].

We will use zeroth order asymptotic expansions

$$\begin{aligned} x(t;\varepsilon) &\sim \bar{x}_0(t) + \bar{X}_0(t/\varepsilon) + \mathcal{O}(\varepsilon), \\ y(t;\varepsilon) &\sim \bar{y}_0(t) + \bar{Y}_0(t/\varepsilon) + \mathcal{O}(\varepsilon), \end{aligned} \tag{19}$$

where $\bar{x}_0(t)$ and $\bar{y}_0(t)$ are the zeroth order regular terms, and $\bar{X}_0(\tau)$ and $\bar{Y}_0(\tau)$ are called boundary functions (or, singular terms). Similar considerations would apply to higher-order

expansions. One can then show, using the homogeneity properties (17), that

$$\begin{array}{l} z(t;\varepsilon,p) \sim \bar{x}_0(t) + \bar{X}_0(pt/\varepsilon) + \mathcal{O}(\varepsilon), \\ w(t;\varepsilon,p) \sim \bar{y}_0(t) + \bar{Y}_0(pt/\varepsilon) + \mathcal{O}(\varepsilon). \end{array} \tag{20}$$

Using the boundary function algorithm formulated in [12, Sect.2.1.2, p.20] one can show that these are asymptotic series, under reasonable regularity assumptions on f and g. We will also assume that the equation $g(x,y,u)=0$ has a unique solution $y=h(x,u)$ for all (x,u) in an open domain of interest. It is easy to show that $\bar{x}_0(t)$ solves:

$$\dot{\bar{x}}_0 = f(\bar{x}_0, h(\bar{x}_0,u(t)),u(t)),\ \bar{x}_0(0)=x_0,\ t\in[0,T]. \tag{21}$$

We will let $y_0(t) := h(\bar{x}_0,u(t))$. Also

$$\bar{Y}_0' = g(x_0,y^*+\bar{Y}_0,u^*) - g(x_0,y^*,u^*),\ \bar{Y}_0(0) = y_0 - \bar{y}_0(0), \tag{22}$$

where $\bar{y}_0(0) = h(x(0),u(0)) = h(x_0,u^*) = y^*$. Finally, one can also show

$$\begin{array}{rcl} \bar{z}_0(t;\varepsilon,p) & = & p\bar{x}_0(t), \\ \bar{w}_0(t;\varepsilon,p) & = & \bar{y}_0(t) + \bar{Y}_0(pt/\varepsilon). \end{array} \tag{23}$$

Using (19) and (20), and recalling the definition (16) of the SI-error $E(t;\varepsilon,p)$, we conclude that the zeroth order approximation $E_0(t;\varepsilon,p)$ of $E(t;\varepsilon,p)$ is:

$$E_0(t;\varepsilon,p) \ = \ E_0(t/\varepsilon;p) \ = \ \bar{Y}_0(pt/\varepsilon) - \bar{Y}_0(t/\varepsilon). \tag{24}$$

Obviously, $E(t;\varepsilon,1) \equiv E_0(t/\varepsilon) \equiv 0$ at $p=1$. Under uniform stability assumptions on g, one can derive, using the theory of asymptotic expansions, an estimate of the following form:

$$\|E_0(\cdot;\varepsilon,p)\|_T \geq M_0, \tag{25}$$

where

$$M_0 = \sup_{\tau\in[0,\infty]} \left\|\bar{Y}_0(p\tau) - \bar{Y}_0(\tau)\right\|, \tag{26}$$

and

$$M_0 \ = \ \mathcal{O}\left(\phi(u_0,u^*)\left|\alpha p - 1\right|(\alpha p)^{\alpha p/(1-\alpha p)}\right) \tag{27}$$

where α is a positive constant independent of ε and p, $\phi(u_0,u^*)$ is a factor that equals zero when $u_0 = u^*$. It follows that $E = E_0 + \mathcal{O}(\varepsilon)$ always satisfies an estimate similar to the one in our simple example (6).

IV. Remarks on stochastic adaptation and scale-invariance

In the analysis of biochemical networks one can proceed with two modeling strategies, a deterministic and a stochastic one [10], [11]. In the deterministic approach, the reaction rate equations are ordinary differential equations, with states being the continuous variables representing the concentrations. A pathway is therefore decomposed into set of elementary reactions, and then the law of mass action is applied to each elementary reaction to obtain the ODEs. However, deterministic models represent an aggregate (mean) behavior of the system, and are not accurate when the "copy numbers" of species (ions, atoms, molecules, individuals) are very small, which is sometimes the case molecular biology at the single-cell level [9].

We have seen that incoherent feedforward motif from Figure 1(b) analyzed in a deterministic setting, was shown to exhibit exact adaptation and an approximate scale invariance. In this section we will informally revisit this example in the light of stochastic adaptation and scale invariance.

The occurrence of chemical reactions in the stochastic setting involves discrete and random events, and in order to predict the progress of chemical reactions in terms of observables such as copy number, $X(t)$, we consider a chemical reaction network consisting of m reactions which involve n species, [11]. We use notation as in [9], and denote the $n\times m$ stochiometry matrix of the network by $\Gamma = \{\gamma_{ij}\}$, $i=1,\dots n$, $j=1,\dots m$, and propensity functions (the rates at which reactions take place) by ρ. The interest is to compute the probability that, at time t there are k_1 units of species S_1, k_2 units of species S_2, k_3 units of species S_3, and so forth:

$$p_k(t) = P[X(t)=k], \tag{28}$$

for each k, where the vector k the state of the process. A chemical master equation (CME) gives a system of linear differential equations for the p_k's. When studying steady state properties we define the steady state distribution $\pi = (\pi_k)$ of the process X as any solution of the equations:

$$\sum_{j=1}^{m} \rho_j(k-\gamma_j)\pi_{k-\gamma_j} - \sum_{j=1}^{m}\rho_j(k)\pi_k = 0\,, \quad k\in\mathcal{Z}^n_{\geq 0}. \tag{29}$$

In order to solve the CME, one usually generates sample paths of the stochastic process $\{X(t)\}$, which is referred to as a stochastic simulation (see e.g. [2]). It can be shown that one can derive exact or approximate differential equations satisfied by the mean and the variance of $X(t)$, [9]. For mass-action kinetics and all reactions of order at most two, the fluctuation-dissipation (FD) formula for the mean $\mu(t) = \mathrm{E}[X(t)]$, and covariance matrix $\Sigma(t) = \mathrm{Var}[X(t)]$ can be derived, see for example [9]. The FD formula is exact for zero and first order mass-action reaction. We next revisit (3) which gives rise to an exact adaptation and an approximate scale invariance, in the light of stochastic adaptation. We additionally provide an example of another feedforward circuit, IFFL2, for which the FD formula is only approximate. Finally, we briefly discuss a minimal stochastically adaptive "two state protein scheme" previously discussed in [4].

A. Adaptation of feedforward model

We study here the model represented by the following reactions:

$$\begin{array}{l} \emptyset \xrightarrow{u} X \xrightarrow{1} \emptyset \\ X \xrightarrow{1/\varepsilon} X + Y\,, \qquad Y \xrightarrow{u/\varepsilon} \emptyset \end{array}$$

The set of ODEs for the deterministic setting are given by $\dot{x} = u - x$, and $\varepsilon\dot{y} = x - uy$, and was previously analyzed.

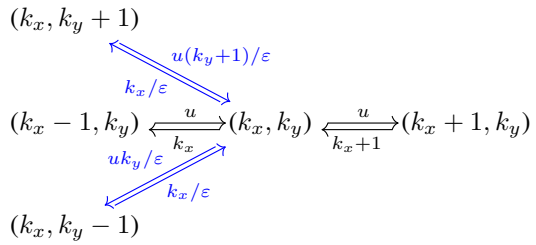

Fig. 3: State transition diagram for an IFFL circuit

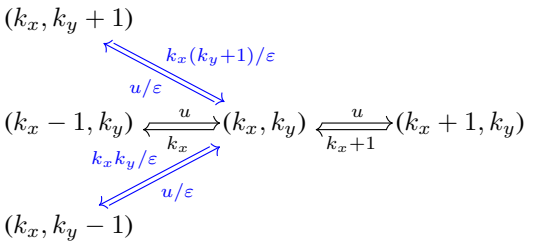

Fig. 4: State transition diagram for an IFFL2 circuit

The state transition diagram corresponding to this circuit is given on Figure 3.

The stochiometry matrix for this system is given by

$$\Gamma = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix},$$

and the propensities for the reactions are $\rho_1(k) = u,\ \rho_2(k) = k_1,\ \rho_3(k) = \frac{1}{\varepsilon}k_1,\ \rho_4(k) = \frac{1}{\varepsilon}uk_2$, where we denote

$$\begin{aligned} R(k) &= \begin{bmatrix} u & X & \frac{X}{\varepsilon} & \frac{1}{\varepsilon}uY \end{bmatrix}^T, \\ f(X,Y) &= \Gamma R(k) = \begin{bmatrix} u - X \\ \frac{1}{\varepsilon}X - \frac{1}{\varepsilon}uY \end{bmatrix}. \end{aligned}$$

The following equations for the means can be obtained:

$$\dot{\mu}_x(t) = u - \mu_x\,, \quad \dot{\mu}_y(t) = \frac{1}{\varepsilon}\mu_x - \frac{u}{\varepsilon}\mu_y. \tag{30}$$

Since all reactions are of order zero or one, the first moments are the same as the deterministic ones. At the steady state $\mu_x^{ss} = u$, and $\mu_y^{ss} = 1$. Hence, the first moment of y (the output) adapts perfectly.

The following system of equations for the covariances can be obtained:

$$\begin{aligned} \dot{\Sigma}_{xx} &= -2\Sigma_{xx} + u + \mu_x\,, \quad \dot{\Sigma}_{xy} = \frac{1}{\varepsilon}\Sigma_{xx} - \frac{u}{\varepsilon}\Sigma_{xy} - \Sigma_{xy}, \\ \dot{\Sigma}_{yy} &= \frac{2}{\varepsilon}\Sigma_{xy} - \frac{2u}{\varepsilon}\Sigma_{yy} + \frac{1}{\varepsilon}\mu_x + \frac{u}{\varepsilon}\mu_y. \end{aligned} \tag{31}$$

We denoted $\mathrm{cov}(X,Y)$ by Σ_{XY}. At the steady state we obtain that:

$$\begin{aligned} \Sigma_{xx}^{ss} &= \frac{u + \mu_x^{ss}}{2} = u, \quad \Sigma_{xy}^{ss} = \frac{\frac{1}{\varepsilon}\Sigma_{xx}^{ss}}{\frac{u}{\varepsilon} + 1} = \frac{u}{u+\varepsilon}, \\ \Sigma_{yy}^{ss} &= \frac{1}{2u}\left(2\Sigma_{xy}^{ss} + \mu_x^{ss} + u\mu_y^{ss}\right) = 1 + \frac{1}{u+\varepsilon} \end{aligned} \tag{32}$$

From (32), we notice that for large u, $u \gg 1$, and small ε, $\Sigma_{yy}^{ss} \approx 1$, which is independent of u. Morever, for $\varepsilon \ll 1$ the system (with output y) also shows approximate scale invariance. To show that this is true, we suppose that $(\mu_x(t), \mu_y(t))$ is any solution corresponding to the input $u(t)$, for the system described by (30). Then, $(p\mu_x(t), \mu_y(t))$ is a solution corresponding to the input $pu(t)$:

$$\begin{aligned} \dot{\mu}_x &= u - \mu_x \quad \Rightarrow (p\dot{\mu}_x) = (pu) - (p\mu_x), \\ \varepsilon\dot{\mu}_y &= \mu_x - u\mu_y\,, \\ \varepsilon &\approx 0 \quad \Rightarrow \quad \mu_y = \frac{p\mu_x}{pu}\,. \end{aligned} \tag{33}$$

Hence the scaling $\mu_x \mapsto p\mu_x$ and $u \mapsto pu$ leaves the μ_y equation (approximately) invariant. Similarly, from (31),

$$\begin{aligned} (p\dot{\Sigma}_{xx}) &= -2(p\Sigma_{xx}) + (pu) + (p\mu_x), \\ \varepsilon\dot{\Sigma}_{xy} &= p\Sigma_{xx} - (pu)\Sigma_{xy} - \varepsilon\Sigma_{xy}\,, \\ \varepsilon\dot{\Sigma}_{yy} &= 2\Sigma_{xy} - 2(pu)\Sigma_{yy} + (p\mu_x) + (pu)\mu_y\,. \end{aligned} \tag{34}$$

The last two expressions in (34) can be estimated by using their quasi-steady state approximation as:

$$\begin{aligned} \varepsilon \approx 0 \ &\Rightarrow\ \Sigma_{xy} \approx \tfrac{p\Sigma_{xx}}{pu} = \tfrac{\Sigma_{xx}}{u}, \\ \varepsilon \approx 0 \ &\Rightarrow\ \Sigma_{yy} \approx \tfrac{\Sigma_{xx}}{pu^2} + \tfrac{1}{2u}\mu_x + \tfrac{1}{2}\mu_y\,. \end{aligned} \tag{35}$$

For large u, the first two terms in the second expression of (35) are negligible, so we finally obtain that under these assumptions the variance of the output y does not depend on the scale p, and the approximate scale invariance (of the mean and the variance of y) can be obtained.

B. *Another feedforward model (IFFL2)*

We also consider the following feedforward model in which the state degrades the output (IFFL2), for which an approximate scale invariance can also be shown in the deterministic setting. The chemical reactions underlying this model are given by:

$$\begin{aligned} &\emptyset \xrightarrow{u} X \xrightarrow{1} \emptyset\,, \qquad \emptyset \xrightarrow{u/\varepsilon} Y \\ &X + Y \xrightarrow{1/\varepsilon} X \end{aligned}$$

In the deterministic setting the model is described by the following ODEs: $\dot{x} = u - x$, $\varepsilon\dot{y} = u - xy$. The state transition diagram corresponding this circuit is given on Figure 4. The stochiometry matrix is

$$\Gamma = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix},$$

and $R(k) = \begin{bmatrix} u & X & \frac{u}{\varepsilon} & \frac{1}{\varepsilon}XY \end{bmatrix}^T$. The means solve:

$$\dot{\mu}_x(t) = u - \mu_x\,, \quad \dot{\mu}_y(t) = \frac{1}{\varepsilon}u - \frac{1}{\varepsilon}\Sigma_{XY} - \frac{1}{\varepsilon}\mu_x\mu_y, \tag{36}$$

It follows from (36) that the equations for the first moment of the output y do not match the corresponding deterministic ODEs. Notice also that the reactions are at most of order two, unlike the previous example where the reactions were at most order one. Hence the expressions for the second moments will only be approximate. At the steady state $\mu_x^{ss} = u$, and

in order to solve the second equation in (36) we need to use the second moment equations:

$$\dot{\Sigma}_{xx} = -2\Sigma_{xx} + u + \mu_x\,, \dot{\Sigma}_{xy} = -\frac{\bar{Y}}{\varepsilon}\Sigma_{xx} - \frac{\bar{X}}{\varepsilon}\Sigma_{xy} - \Sigma_{xy},$$
$$\dot{\Sigma}_{yy} = -\frac{2\bar{Y}}{\varepsilon}\Sigma_{xy} - \frac{2\bar{X}}{\varepsilon}\Sigma_{yy} + \frac{u}{\varepsilon} + \frac{1}{\varepsilon}(\Sigma_{xy} + \mu_x\mu_y).$$

At the steady state,

$$\begin{aligned} &\mu_x^{ss} = u\,, \quad \mu_y^{ss} = 1 + \frac{\frac{\bar{y}}{\varepsilon}}{\frac{\bar{x}}{\varepsilon}+1}\,, \\ &\Sigma_{xx}^{ss} = u\,, \quad \Sigma_{xy}^{ss} = \frac{\frac{-\bar{y}u}{\varepsilon}}{\frac{\bar{x}}{\varepsilon}+1}\,, \quad \Sigma_{yy}^{ss} = \frac{\frac{\bar{y}^2 u}{\varepsilon}}{\frac{\bar{x}^2}{\varepsilon}+\bar{x}} + \frac{u}{\bar{x}}, \end{aligned} \tag{37}$$

and $\bar{x}$ and $\bar{y}$ could be chosen to be (i) equal to the deterministic means for $x(t)$ and $y(t)$, or (ii) solved for using the stochastic means.

The results presented so far are summarized in Table I.

	y_{det} adapts	μ_y adapts	Σ_{yy} adapts	FD exact
IFFL1	yes	yes	no	yes
IFFL2	yes	no	no	no

TABLE I: Summary of adaptation results in a stochastic and a deterministic setting; y_{det} denotes the deterministic solution for y, μ_y and Σ_{yy} are its mean and variance. FD exact means that the differential equations for the first two moments are exact.

C. The two state protein model

To identify a minimal network that adapts, we modify the example discussed in [4].

$$\emptyset \xrightarrow{k} Y \xrightarrow{\alpha} \emptyset\,, \qquad Y \xrightarrow{u/\varepsilon} Z$$
$$Z \xrightarrow{c/\varepsilon} Y$$

The stochiometry matrix for this system is

$$\Gamma = \begin{bmatrix} 1 & -1 & -1 & 1 \\ 0 & 0 & 1 & -1 \end{bmatrix},$$

and $R(k) = \begin{bmatrix} k & \alpha Y & \frac{u}{\varepsilon}Y & \frac{c}{\varepsilon}Z \end{bmatrix}^T$. The means solve:

$$\begin{aligned} \dot{\mu}_y(t) &= k - (\alpha + \frac{u}{\varepsilon})\mu_y + \frac{c}{\varepsilon}\mu_z, \\ \dot{\mu}_z(t) &= \frac{u}{\varepsilon}\mu_y - \frac{c}{\varepsilon}\mu_z, \end{aligned} \tag{38}$$

where Y is the output of interest. At the steady state $\mu_y^{ss} = \frac{k}{\alpha}$, and $\mu_z^{ss} = \frac{uk}{\alpha c}$. Then the problem simplifies to solving

$$\begin{aligned} \dot{\Sigma}_{yy} &= -2\Sigma_{yy}(\alpha + \frac{u}{\varepsilon}) + 2\frac{c}{\varepsilon}\Sigma_{yz} + k + \alpha\mu_y + \frac{u}{\varepsilon}\mu_y + \frac{c}{\varepsilon}\mu_z \\ \dot{\Sigma}_{yz} &= \frac{u}{\varepsilon}\Sigma_{yy} - \Sigma_{yz}(\frac{c}{\varepsilon} + \alpha + \frac{u}{\varepsilon}) + \frac{c}{\varepsilon}\Sigma_{zz} - \frac{u}{\varepsilon}\mu_y - \frac{c}{\varepsilon}\mu_z \\ \dot{\Sigma}_{yy} &= \frac{2u}{\varepsilon}\Sigma_{yz} - \frac{2c}{\varepsilon}\Sigma_{zz} + \frac{u}{\varepsilon}\mu_y + \frac{c}{\varepsilon}\mu_z \end{aligned}$$

At the steady state the system simplifies to

$$\Sigma_{yy} = \frac{c\Sigma_{yz}}{\alpha\varepsilon + u} + \frac{k}{\alpha}\,, \quad \Sigma_{zz} = \frac{u\Sigma_{yz}}{c} + \frac{uk}{\alpha c}\,, \quad \Sigma_{yz} = 0.$$

Hence,

$$\Sigma_{yy} = \frac{k}{\alpha}\,, \quad \Sigma_{zz} = \frac{uk}{\alpha c}\,, \quad \Sigma_{yz} = 0.$$

Since y was taken as the output to the system, we notice that the variance of the output also adapts. Moreover if $k = c = \alpha = 1$ then $\Sigma_{yy} = 1$, $\Sigma_{zz} = u$, $\Sigma_{yz} = 0$.

V. CONCLUSIONS

Experimental observations of scale-invariance have given rise to follow-up modeling and theoretical research aimed at analyzing systems that display the SI property. We have discussed the existence of a fundamental limitation of a mechanism based on time-scale separation between internal variables and output variables, for scale-invariance, showing that there is a minimal finite error that cannot be overcome, no matter how large the separation of time scales is.

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