Design principles for perfect adaptation in biological networks with nonlinear dynamics

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Abstract

Establishing a mapping between the emergent biological properties and network structure has always been of great relevance in systems and synthetic biology. Adaptation is one such biological property of paramount importance, which aids in regulation in the face of environmental disturbances. In this paper, we present a nonlinear systems theory-driven framework to identify the design principles for perfect adaptation in the presence of large disturbances. Based on the input-output configuration of the network, we use invariant manifold methods to deduce the condition for perfect adaptation to constant input disturbances. Subsequently, we translate these conditions to necessary structural requirements for adaptation in networks of small size. We then extend these results to argue that there exist only two classes of architectures for a network of any size that can provide local adaptation in the entire state space—namely, incoherent feed-forward structure and negative feedback loop with buffer node. The additional positiveness constraints further restrict the admissible set of network structures—this also aids in establishing the global asymptotic stability for the steady state given a constant input disturbance. The entire method does not assume any explicit knowledge of the underlying rate kinetics barring some minimal assumptions. Finally, we also discuss the infeasibility of the incoherent feed-forward structure to provide adaptation in the presence of downstream connections. Our theoretical findings are corroborated by detailed and extensive simulation studies. Overall, we propose a generic and novel algorithm based on a nonlinear systems theory to unravel the design principles for global adaptation.

1 Introduction

Systems biology has emerged as an important pillar of biology over the last two decades. A key aspect of systems biology is the understanding of network structure and function—this essentially leads to the adoption of either a conventional systems approach or a graph network formalism. The knowledge of the constitutive units (typically represented as nodes in a biochemical network) can be obtained from the traditional disciplines of biology. Further, it is widely believed from increasing shreds of evidence that the network architecture for a given *phenotype* remains conserved across the organism space.[1,2] Therefore, given a sufficient understanding of these constitutive units, finding the appropriate manner of interconnections *i. e.* *design principles* for important functionalities remains an exciting area of study that has attracted multi-disciplinary scholarship.

Adaptation, a ubiquitous mechanism in every living organism, is one such property that has been of sustained interest in the broader community of science. Adaptation serves a crucial role in a variety of processes ranging from bacterial chemotaxis to mammalian homeostasis of important metabolites. Typically, adaptation involves two subsequent steps–1) sensing the external disturbance and 2) reverting to its pre-disturbed level. Therefore, evaluating the performance of a system vis-a-vis adaptation requires performance parameters that cater to both the aforementioned steps. For this purpose, Ma *et al* (2009) proposed two parameters, namely *sensitivity* and *precision*.[1] A perfectly adaptive system should provide infinite precision with a non-zero but finite sensitivity. Fig. 1 provides a schematic of a typical adaptive response and the associated formula for sensitivity and precision.

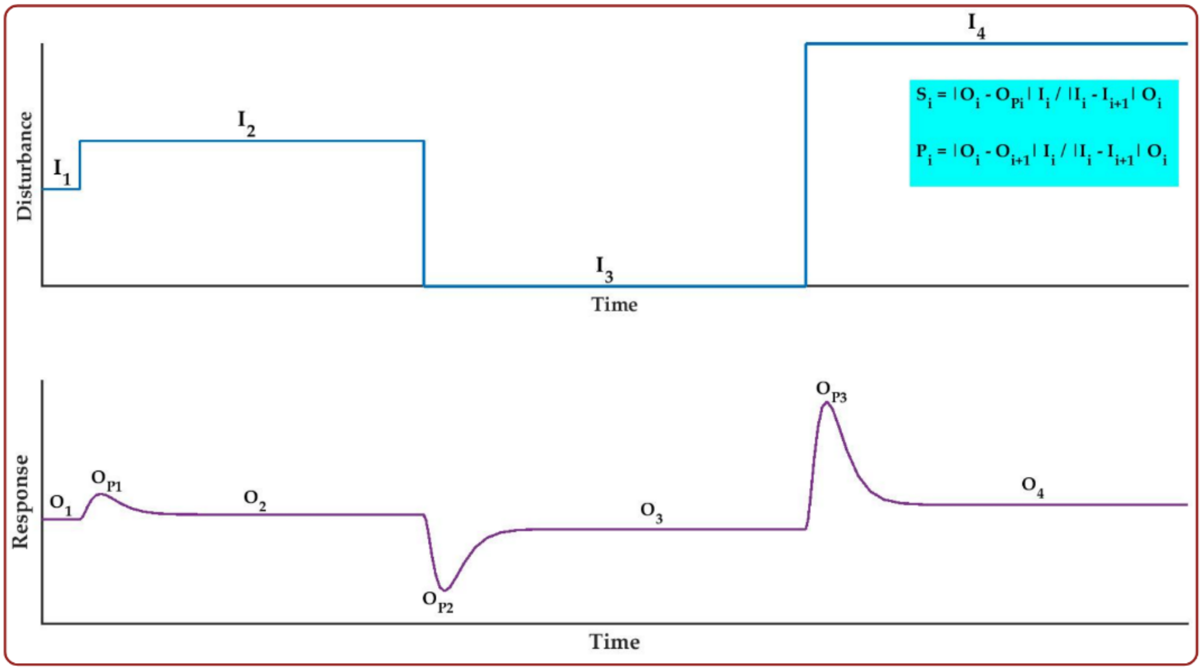

Figure 1: An example of adaptation response for stair-case/multi-step disturbance input. From the expression shown in the figure, it is clear that precision (P) and sensitivity (S) has to be infinite and non-zero finite respectively for each step change.

Identifying network structures capable of adaptation has attracted a lot of multi-disciplinary attention ranging from computational sciences to mathematical systems theory, thereby giving rise to broadly three different categories of approaches.[3] As the name suggests, the computational screening approach involves scanning through the entire topology parameter sets. Previously, Ma *et al* (2008) adopted a three-protein network and scanned through every possible topology-parameter combination with an assumption of Michaelis-Menten rate kinetics. Interestingly, it was found that all the adapting network structures contain either a negative feedback loop with buffer action or multiple forward paths from the disturbance-receiving node to the output node with mutually opposite effects.[1] Subsequently, Qiao *et al* (2019) extended the framework to a stochastic scenario where the input disturbance was assumed to be stochastic. Apart from identifying the adaptive structures, a pair-wise correlation study between the performance parameters such as sensitivity, precision, and output Signal to Noise Ratio (SNR) was carried out to obtain network structures capable of accomplishing the dual task of adaptation and noise filtering. It was found that for small scale-network structures, the correlation between the output SNR and sensitivity is negative, indicating an incompatibility between perfect adaptation and noise attenuation. However, it is possible to achieve both qualities if the dedicated adaptation and noise filtering modules are connected in series.[4] Apart from the aforementioned simulation-based techniques, there exist optimization-based algorithms that aim to find out adaptive network structures by solving a mixed nonlinear integer programming-based optimization techniques.[5]

On the other hand, specific design strategies inspired by human-made systems have also been adopted to construct adaptive biochemical networks. Most of these design rules have been inspired by the seminar work by E. Sontag (2004) where it was argued that biological adaptation, in essence, is a disturbance rejection problem.[6] Therefore, from the celebrated internal model principle in control theory, a system capable of rejecting a step-type disturbance should contain an integrator either in its controller or plant module. Inspired by this Briat *et al* (2016) proposed an integral controller-based design, namely antithetic integral controller (AIC), that can provide perfect adaptation for constant disturbances in a stochastic setting.[7] Subsequently, it was observed that the AIC-based design produces perfect adaptation at the cost of increased variance. An additional negative feedback was recommended over and above the one customary to the controller module to circumvent the overshoot of variance,.[8]

The systems-theoretic approach begins with defining a few performance parameters characteristic of biological adaptation. When mapped to the standard parameters of the underlying dynamical system (such as poles, zeros, gain *etc.*), these performance parameters give rise to a number of abstract mathematical conditions for adaptation. Further, using algebraic graph theoretic strategies, these abstract conditions are translated into structural requirements for adaptation.[3,9] Previously, it has been shown in several works that perfect adaptation – infinite precision and non-zero finite sensitivity translates to a zero-gain system condition in the linear, time-invariant dynamical system.[10–13] Bhattacharya *et al* (2018) used these systems-theoretic conditions to provide the network structures for perfect adaption for disturbance of small magnitude in three node networks.[13] Subsequently, Araujo *et al* (2018) and Wang *et al* (2019, 2021) adopted a graph-theoretic approach to provide the structural requirements of perfect adaptation in the presence of small disturbances for a network of any size.[14–16] It has been argued that a network, irrespective of its size, must contain either a *balancer* or an *opposer* module to provide perfect adaptation in the presence of step-type deterministic disturbance of small magnitude. Further, Araujo *et al* conjectured that the balancer modules should contain at least one negative feedback loop for the purpose of stability.[14] Recently, Bhattacharya *et al* (2022) proved the conjecture and proposed additional structural requirements to obtain the strictest necessary conditions for perfect adaptation.[17]

The computation screening, albeit a beneficial starting point, remains computationally burdensome, thereby compromising on the *scalability* of the results. Further, the computational approaches require explicit knowledge about the rate laws for examining the structural possibilities. Therefore, the conclusions drawn from these studies remain primarily confined to the assumed kinetics, thereby losing out on *generalizability*. The rule-based or specific design strategies circumvent both the issues but at the cost of being able to detect all the possible structural combinations for adaptation – indicating a possible loss of *exhaustivity*. On the other hand, the systems-theoretic approaches, albeit their impressive strides vis-a-vis the scalability, generalizability, and exhaustivity, have primarily assumed the input disturbance to be small enough. Additionally, a few common assumptions that run through the preceding contributions in systems-theoretic approaches are– i) the input disturbance is of small magnitude, and ii) the underlying dynamical system is assumed to be relaxed prior to the step-type disturbance. The assumption of the input magnitude is small enough such that the states are not pushed into an unstable region (or beyond the domain of attraction of the stable operating point) allowed to proceed with a linearised treatment of the problem. The second assumption translates to the requirement that the interval between two subsequent input step-type disturbances is larger than the system's settling time. None of the aforementioned assumptions can be ensured in reality. On the other hand, the real-life network structures that have been obtained through experiments not only form a small subset of ones derived via the linearised treatment but also are capable of regulating the output for an astonishingly wide range of input disturbances.[18,19] Although there have been several interesting discussions about the scale or translation-invariance of biological networks that have proposed novel insights into building a nonlinear systems-driven framework for disturbance rejection extending it to structural requirements remains an open task.[20,21]

In this work, we present a nonlinear systems theory-driven framework to identify the design principles for *global, perfect adaptation* in the presence of *deterministic* disturbance of arbitrary magnitude (GPAD). The adaptation capability in the global sense (anywhere in the state space) relaxes the assumptions mentioned above in the linearised treatment. Further, the nonlinear treatment renders the imposition of practical constraints on the system's treatment of biological adaptation possible. For instance, since all the states are considered as the concentration of the biological species such as proteins, genes it is customary for the resulting dynamical system to be *positive*,[22] a condition that can not be accommodated in a linearized system's framework. To check the veracity of the framework, we first use the conditions inspired by nonlinear systems theory to obtain the topologies for perfect adaptation in a localized sense. Conceivably, the resultant network structures obtained for local analysis coincides with the structural predictions obtained using a linearised analysis. Hence, the proposed method is unique in literature, for it does not entirely rely on the Jacobian analysis of the system to provide the admissible topologies for adaptation in the presence of small input disturbance. Further, we utilize the conditions for positiveness and global stability to deduce the network structures that can provide perfect adaptation irrespective of the amplitude and time interval of the step-type disturbances.

The paper is organized in the following manner. Section 2 outlines the methodology that is used to arrive at precise mathematical conditions for GPAD. Subsequently, section 3 entails the application of the proposed methodology in determining the structural requirements for GPAD in biochemical networks. Finally, section 4 attempts to put all the results in perspective and tally the contributions vis-a-vis the existing literature.

2 Methodology

This section presents a generic framework inspired by nonlinear systems theory to deduce the conditions for adaptation. These conditions can then be applied to biological networks to determine candidate network structures for adaptation.

Given a biochemical network with the concentration vector of the biochemical species denoted as $\mathbf{x}$, the underlying dynamical system formulated from the reaction kinetics can be written as

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}, \mathbf{u}) \tag{1}$$

$$\mathbf{y}(t) = \mathbf{h}(\mathbf{x}, \mathbf{u}) \tag{2}$$

where, $\mathbf{u}(t)$ is the external input species of the network. Further, the dynamics of the input node can be expressed as

$$\dot{\mathbf{u}} = f_u(\mathbf{u}(t)) \tag{3}$$

It can be seen that (1) and (3) together form a *triangular* dynamical system. According to the stability theorems on triangular systems,[23] the system of the form

$$\dot{\mathbf{u}} = f_u(\mathbf{u}), \qquad \dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}, \mathbf{u})$$

is Lyapunov stable at the origin if and only if $\mathbf{u}(t)$ is Lyapunov stable and $\mathbf{x}$ has autonomous asymptotically stable dynamics around the origin.

2.1 Assumptions

Here we list out important assumptions on the network dynamics, which shall be valid throughout the text unless specified otherwise.

1. For an $N-$node protein system with concentrations x_i's $(i = 1, 2, \cdots, n)$ the dynamics of the concentration of k^{th} protein is assumed to be of the form

$$\dot{x}_k = \sum_{j=1}^{N} f_{kj}(x_k, x_j) \tag{4}$$

2. The flow associated with (4) satisfies semi-group property and Lipschitz continuous with respect to the states.

3. The system of differential equations constructs a well-posed system.

4. If $i \neq j$ then for a given x_i, $|f_{i,j}(x_i, x_j)|$ is class $\mathcal{K}$ function with respect to x_j *i. e.* $f_{i,j} = 0$ at $x_j = 0$ and monotonic function of x_j in the closed interval $(0, x_{j_{max}})$.

5. $|f_{i,j}(x_i, x_j)|$ is monotonically decreasing with respect to x_i in the event of activation and increasing for repression.

6. In the case of activation, $|f_{i,j}(x_i, x_j)| = 0$ when $x_i = x_{i_{\max}}$ and $\arg\min_{x_i} f_{i,j}(x_i, x_j) = x_{i_{\min}}$. Similarly, in the case of repression, $|f_{i,j}(x_i, x_j)| = 0$ when $x_i = x_{i_{\min}}$ and $\arg\max_{x_i} |f_{i,j}(x_i, x_j)| = x_{i_{\max}}$

7. The input disturbance (Denote it as v) is only connected to one protein (denote it as x_1), and the effect is mediated through inter-protein connections. Further, the connection is reflected in the following way.

$$\dot{x}_1 = f_1(\mathbf{x}) + f_{1v}(x_1)v$$

These assumptions aid in guaranteeing stability and perfect output regulation for a wide range of biologically relevant rate dynamics.

2.2 Conditions for perfect adaptation

In this subsection, we derive the main conditions for any biochemical network to produce the perfect adaptation for bounded input with zero dynamics.

Consider a biochemical network with N proteins. Without any loss of generality, let us denote the concentration of the input-receiving node as x_1. Further, the output is measured as the concentration of the k^{th} node. Given concentration vector $\mathbf{x} \in \mathbb{R}^N$ as state variables and v as the disturbance input, the underlying dynamics of the network can be written as

$$\dot{v} = f_v(v) \tag{5}$$

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}) + \mathbf{f}_v(\mathbf{x})v \tag{6}$$

$$y(t) = \mathbf{e}_k^T \mathbf{x} \tag{7}$$

where, $\mathbf{e}_k \in \mathbb{R}^N$ is the unit vector in the direction of x_k and given,

$$\left.\frac{\partial f_v(v)}{\partial v}\right|_v^* = 0 \tag{8}$$

As discussed earlier, for perfect adaptation, the required non-zero, finite sensitivity can be guaranteed if the system is controllable by the disturbance input v *i. e.* it should satisfy the following condition

$$\mathbb{D} := span\left\{\mathbf{f}, \mathbf{f}_v, [\mathbf{f}, \mathbf{f}_v], [\mathbf{f}, [\mathbf{f}, \mathbf{f}_v]], [\mathbf{f}_v, [\mathbf{f}, \mathbf{f}_v]] \cdots \right\} = \mathbb{R}^N \tag{9}$$

where, $[\mathbf{t}, \mathbf{p}]$ refers to the Lie brackets between two vectors $\mathbf{t}$ and $\mathbf{p}$ in the tangent space of the manifold the state space evolves on.[24]

Similarly, the infinite precision condition can be perceived as the invariance of the steady state level of the output with respect to the disturbance input. Therefore, perfect adaptation can be guaranteed if there exists a steady state $(v^*, \mathbf{x}^*(v))$ in R^{N+1} such that

$$\mathbf{f}_v(v^*) = 0 \tag{10}$$

$$\mathbf{f}(\mathbf{x}(v^*)) + \mathbf{f}_v(\mathbf{x}(v^*), v^*) = 0 \tag{11}$$

$$\text{and, } x_k(v^*) = k_0 \text{ (constant)} \tag{12}$$

Further, the steady state must be Lyapunov stable for perfect adaptation to non-trivial initial conditions. For this purpose, we adopted a two-step procedure where in the first step, we guarantee the local stability of the system by verifying whether the Jacobian matrix at a particular steady state is stable. If yes, subsequently, we investigate whether there exists a compact, positive invariant set (Ω) containing only the concerned steady state. For this purpose, we use Nagumo's theorem (1946),[25] which provides the necessary methodology to check whether a given set is positive invariant.

Nagumo's Theorem (1943) 1. *Assume the dynamical system in* (1) *has a unique solution for a constant* u. *Consider the set* $\mathcal{C} : \mathbf{x} \in \mathbb{R}^N, \mathcal{C}_i(\mathbf{x}) \leq \mathbf{b}$ *where,* $\mathcal{C}_i(\mathbf{x})$ *and* $\mathbf{b}$ *are smooth functions of* $\mathbf{x}$ *such that* $\Delta\mathcal{C}_i(x) \neq 0 \ \forall \mathbf{x} \in \partial\mathcal{C}$ *and constant column vector respectively. Further, if the set containing the active constraints is only non-empty on the boundary, then the closed set* $\mathcal{C}$ *is positive invariant with respect to* (1) *if and only if*

$$\Delta\mathcal{C}_i(x)\mathbf{f}(\mathbf{x}, u) \leq 0, \forall \mathbf{x} \in \partial\mathcal{C} \textit{ and } i \in \mathbb{A}(\mathbf{x})$$

where, $\mathbb{A}(\mathbf{x})$ *is the set of active constraints at the boundary of* $\mathcal{C}$.

Finally, using a Lyapunov function (V) in Ω we show that for adaptation capable networks $\dot{V}(\mathbf{x}) \leq 0$, $\forall \mathbf{x} \in \Omega$, the equality holds only at $\mathbf{x} = \bar{\mathbf{x}}(v)$, where $\bar{\mathbf{x}}(v)$ is the isolated unique steady state of (6). Therefore, from LaSalle's invariance principle, we conclude $\bar{\mathbf{x}}(d)$ is a globally asymptotically stable steady state for a given disturbance level.

Interestingly, it can be seen that the scenario of perfect adaptation in the neighborhood of the steady state *i. e. infinitesimal adaptation*, as termed by[16] satisfies the premise of the celebrated central manifold theorem (CMT).[26] In that case, Eq. 7-9 serves as the CMT equations, which can be formulated as the conditions for perfect infinitesimal adaptation and the localized stability requirement.

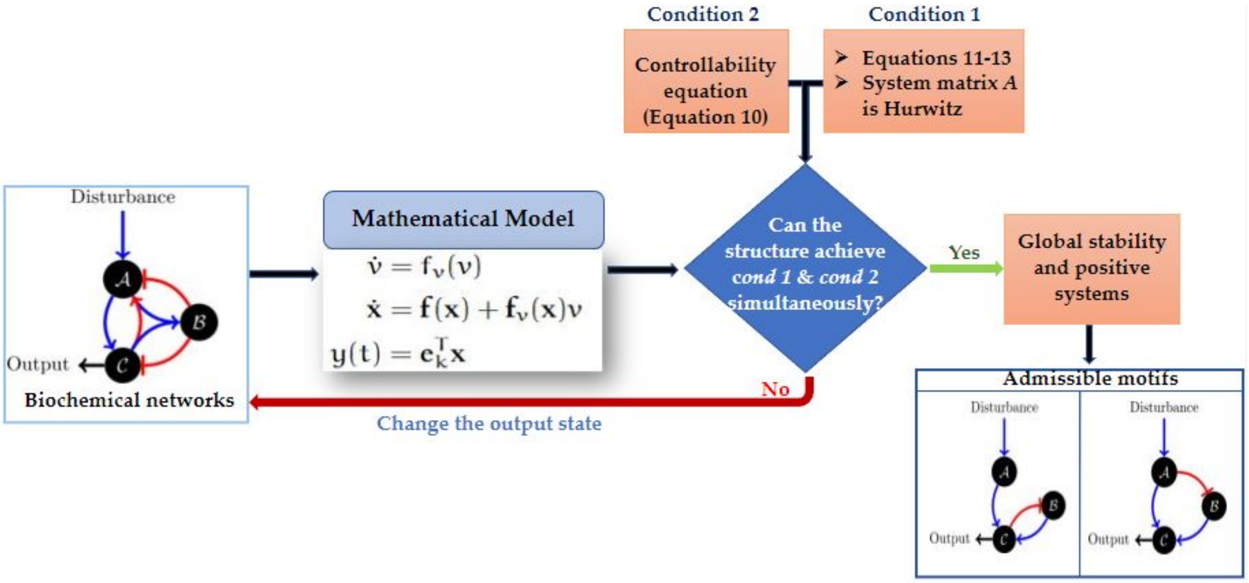

Figure 2: **Workflow of the proposed methodology.** Any given protein network is first linearized, and the conditions on the **A** matrix are investigated to ultimately derive admissible motifs for the desired functionality.

3 Results

We use this section to present the novel insights gained by applying the proposed methodology in Figure 2 on the biochemical networks. The nodes characterize a biochemical network as the biochemical species and the edges as the interactions. Further, as defined in the preceding chapters, each edge in the network can be of two types– activation or repression leading to the emergence of many structural possibilities. Although the question of establishing global stability for biochemical networks has been attempted in considerable detail, the approach has been limited to either a mass-action or synergy kinetics[27] or a particular type of dynamics that assumes a radial unboundedness of the rate kinetics for a particular state.[28] Therefore, the present intervention attempts to close the stability analysis by focusing explicitly on the networks with rational rate functions, including Michaelis-Menten or Hill kinetics and their variants.

We begin with deriving network structures that can adapt perfectly to an input disturbance of small magnitude. Additionally, we assume that two subsequent step-type disturbances are further than the settling time of the dynamical system. To this purpose, we adopt a bottoms-up approach wherein we first attempt to find the minimal motifs (both nodes and edges) that can perform perfect adaptation.

3.1 Two node networks

As can be inferred from the figure, adaptation is a non-monotonic response. Therefore, the possibility of achieving perfect adaptation from a single-node network (single-state system) can be safely ruled out. The immediate scenario of the two-node network can be examined.

Proposition 1. *A 2-node network can perfectly adapt to constant disturbance if and only if it contains negative feedback and a buffer action at the non-input-receiving state.*

Proof. We shall prove it through contradiction. We first start with a two-node network where the concentration of the input-receiving node ($\mathcal{A}$) is denoted as $x_1(t)$ and the same for the other node ($\mathcal{C}$) is $x_2(t)$. Given $x_2(t)$ is the output node, the corresponding dynamical system can be written as

$$\begin{aligned}\dot{v} &= f_v(v)\\ \dot{x}_1 &= f_{11}(x_1, x_1) + f_{12}(x_1, x_2) + f_{1v}(x_1, v)\\ \dot{x}_2 &= f_{21}(x_2, x_1) + f_{22}(x_2, x_2)\\ y(t) &= x_2(t)\end{aligned}$$

Since the present work focuses on constant (step or staircase type) disturbances, we shall limit our discussion to the case $f_v(v) = 0$.

$$f_{11}(x_1(v), x_1(v)) + f_{12}(x_1(v), x_2(v)) + f_{1v}(x_1(v), v) = 0 \tag{13}$$

$$f_{21}(x_2(v), x_1(v)) + f_{22}(x_2(v), x_2(v)) = 0 \tag{14}$$

Again, according to the condition in (12), replacing the output state $x_2(v)$ with a constant (k_0) we obtain

$$f_{11}(x_1(v), x_1(v)) + f_{12}(x_1(v), k_0) + f_{1v}(x_1(v), v) = 0 \tag{15}$$

$$f_{21}(k_0, x_1(v)) + f_{22}(k_0, k_0) = 0 \tag{16}$$

$$\implies x_1(v) = g(k_0) \tag{17}$$

$$\implies k_0 = \zeta(v) \text{ (Contradiction)} \tag{18}$$

The trivial scenario of feed-forward structure can be safely ruled out as it can be easily shown similarly that the steady-state, in this case, is dependent on the disturbance input.

As it can be inferred from (15) the only way to satisfy (11) and (12) is not to have any edge from the input-receiving node (x_1) to the output node (x_2). However, it can be shown (Supporting information) that this shall render the system uncontrollable, failing to satisfy the condition 9 for perfect adaptation. Therefore, it can be concluded that a two-node network with an output node different from the input-receiving node can not produce the adaptation.

Interestingly, if x_1 is considered as the output node *i. e.* $x_1(v) = k_1$ (constant), the infinite precision condition for adaptation can be written as

$$f_{11}(k_1, k_1) + f_{12}(k_1, x_2(v)) + f_{1v}(k_1, v) = 0 \tag{19}$$

$$\implies x_2(v) = g_1(v) \tag{20}$$

$$f_{21}(g_1(v), k_1) + f_{22}(g_1(v), g_1(v)) = 0 \tag{21}$$

Equation (21) can only be achieved if f_2 is made independent of x_2, which is possible for a class of rate kinetics prevalent in biochemical systems. Further, the corresponding condition for local stability requires the system matrix for the linearised system is Hurwitz. It can be shown that (refer to supporting information) this is possible if node 1 (x_1) and node 2 (x_2) engage in a *negative* cycle. ☐

3.2 Three node networks

The two-node networks, as discussed before, can only provide perfect adaptation when the input-receiving node is considered the output. To circumvent this problem, we conceived a controller node ($\mathcal{B}$) with the following strategy

$$\dot{x_3} = f_{3,1}(x_3, x_1) + f_{3,2}(x_3, x_2) + f_{3,3}(x_3, x_3) \tag{22}$$

$$u = x_3 \tag{23}$$

In the case of the minimal network (not more than three edges), we consider that the controller receives the information about the manipulated state through a single reaction and actuates the control signal through another chemical reaction. Therefore, they can be four such elementary network structures possible.

Proposition 2. *A three-node network can provide local, perfect adaptation if it contains a negative feedback loop that engages a controller node acting as a buffer.*

Proof. Similar to the proof for Proposition 1 ☐

At this juncture, a three-node network can also have a feed-forward structure. We investigate the scenario via the following proposition.

Proposition 3. *Three-node feed-forward network structures can provide local, perfect adaptation if and only if multiple forward paths exist from the input-receiving to the output node with mutually opposite effects.*

Proof. For possible scenario where, $\mathcal{A} \to \mathcal{B}$, $\mathcal{B} \to \mathcal{C}$, $\mathcal{A} \to \mathcal{C}$, the adaptation conditions (in accordance with (11) and (12)) can be written as

$$\begin{aligned} f_{11}(x_1(v), x_1(v)) + f_{1v}(x_1(v), v) &= 0 \\ \implies x_1(v) &= g_1(v) \\ f_{21}(k_0, x_1(v)) + f_{22}(k_0, x_2(v)) + f_{23}(k_0, x_3(v)) &= 0 \\ f_{31}(x_3(v), k_0) + f_{33}(x_3(v), x_3(v)) &= 0 \\ \implies x_3(v) &= g_3(x_1(v)) \end{aligned}$$

Let the change in the disturbance level is v_1 to v_2. Denote the resultant change in f_{ij} be Δf_{ij}. The modified condition for adaptation can be written as

$$\Delta f_{23}(\Delta x_3) + \Delta f_{21}(x_1, \Delta x_1) = 0 \tag{24}$$

$$\implies \Delta f_{23} \circ \Delta g_3(x_1, \Delta x_1) + \Delta f_{21}(x_1, \Delta x_1) = 0 \tag{25}$$

Due to the class $\mathcal{K}$ nature of $|f_{ij}|$ with respect to x_j, x_1, for this structure, possesses a monotonic relationship with v. Therefore, if $\Delta v := v_2 - v_1 > 0 \implies \Delta \geq 0 \implies \Delta |f_{21}| > 0$ owing to the class $\mathcal{K}$ nature of f_{21}. Therefore, the only way to satisfy equation (25) is to have a *mutual opposition* between the edge from $\mathcal{A}$ to $\mathcal{C}$ and the forward path $\mathcal{A} \to \mathcal{B} \to \mathcal{C}$. Fig. 3 responds to a locally adaptive module that possesses multiple forward paths with mutually opposite effects on the output node. □

It is to be observed that in the first three scenarios, the final network structure contains

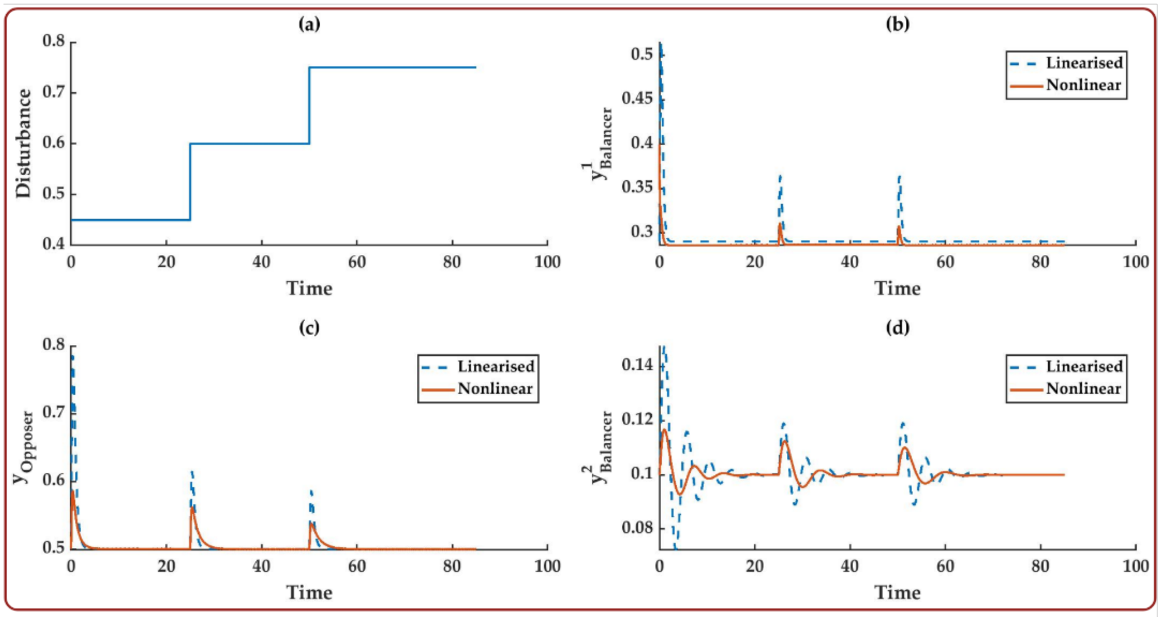

Figure 3: Validates the claims made in Propositions 2 and 3. The relaxed networks provide a perfectly adaptive response in the presence of an external input disturbance of small amplitude. Similar responses can also be obtained from the linearised analysis, which, in this case, remains valid due to small input perturbation.

negative feedback, along with the controller dynamics being independent of the present concentration of the controller species. This class of network structures is termed as *negative feedback loop with buffer node* (NFBLB). Unlike the NFBLB, the end structures in the fourth scenario entail two mutually opposing forward paths from the input to the output node, which is termed as the *incoherent feed-forward with proportional node* (IFFLP).

3.3 General structural requirements for perfect, non-infinitesimal adaptation

The analysis of two and three-node networks in the preceding subsections provides only the minimal networks acceptable for perfect adaptation in a localized sense. At the same time, these results provide a solid foundation to generalize the structural recommendations to networks of any size.

Theorem 1. *For a network with $N-$nodes, it requires at least $N-$edges to provide local, perfect adaptation.*

Proof. Consider an $N-$node network with the concentration of each node species being the state variable. Without any loss of generality, let us denote the concentration of the input-receiving node as $x_1(t)$.

We shall attempt to prove this by contradiction. It is trivial that an $N-$node network is structurally controllable only if it contains $\geq N-1$ links.[29] The resultant network structure, in this case, can not contain any loops to ensure structural controllability. Further, each node must be connected to the input-receiving node by one forward link. Therefore, the resultant graph shall always be isomorphic to a *hub and spoke* network, with the hub being the input-receiving node. Furthermore, there exists no path between the nodes of two different spokes. Suppose the output node is situate at the k^{th} spot of p^{th} spoke. Therefore, the perfect adaptation condition for the nodes in that branch.

$$\dot{v} = 0 \tag{26}$$

$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \vdots \\ \dot{x}_k \\ \dot{x}_{k+1} \\ \vdots \end{bmatrix}_{\mathbf{x}=\mathbf{x}^*} = \begin{bmatrix} f_{11}(x_1) + f_{1v}(x_1, v) \\ f_{21}(x_2, x_1) + f_{22}(x_2) \\ \vdots \\ f_{kk-1}(x_k, x_{k-1}) + f_{kk}(x_k) \\ f_{k+1k}(x_{k+1}x_k) + f_{k+1k+1}(x_{k+1}) \\ \vdots \end{bmatrix}_{\mathbf{x}=\mathbf{x}^*} = \mathbf{0} \tag{27}$$

Further, putting $x_k^* = k_0$ (constant) for infinite precision, we obtain from Eq. (27) $x_k^* = k_0$ $\implies$ $x_1 = k_1$ $\implies$ $v = f(k_1) = k_2$ where k_0, k_1 and k_2 are constants – This contradicts the assumption that v is external. Therefore a network with N nodes requires at least N edges to perform global, perfect adaptation. □

Biochemical networks can be divided into two groups, namely, i) feed-forward network and ii) networks with loops. Our framework investigates these two categories of networks individually to deduce the specific signature connections for perfect adaptation.

Theorem 2. *An N-node controllable (by the input-receiving node) network without any loops performs perfect adaptation if there are multiple forward paths from the input-receiving node to the output node and at least a pair of them have mutually opposite effects on the output node.*

Proof. Given an N-node feed-forward network, the external input is connected to the node $x_1(t)$. The maximum number of nodes $x_1(t)$ can influence is $N-1$. It is to be noted that for structural controllability, there exists at least one forward path from x_1 to every other node in the network. Hence, there is no incoming edge possible at x_1 in a feed-forward network.

Let us consider the set $\mathcal{N}_1$ ($\mathfrak{K}(\mathcal{N}) = k_1$, where $\mathfrak{K}$ refers to cardinality) consists of all the nodes that contain an incoming edge from the node x_1. These nodes are connected to the downstream networks. We define the reachable set $\mathcal{R}_j$ at a node x_j as the set of all nodes with an incoming edge from x_j. Further, $\mathcal{F}_j$ contains all the forward paths from x_1 to x_j. For instance, if a three-node network contains two forward paths from x_1 to x_3 such as $x_1 \to x_2 \to x_3$ and $x_1 \to x_3$ then

$$\mathcal{F}_3 = \{(x_1, x_2), (x_1)\}$$

For a feed-forward network without any loops, it can be proved (Supporting information) using steps similar to Theorem 1 that nodes that have only a single incoming path from node x_1 (hence the input disturbance) can not produce the perfect adaptation. Therefore, a given node x_j should be considered for adaptation if

$$\mathfrak{K}\Big(\mathcal{F}_j\Big) \geq 1 \tag{28}$$

This is only possible if x_j is a common member of the reachable sets of at least two nodes (denote, x_i and x_k) provided the ordered set (x_i, x_j) or (x_j, x_i) does not belong to any of the sets in $\mathcal{F}_j$. Evidently, the consideration of two nodes is without any loss of generality.

Suppose the node x_j satisfies (28) *i. e.* there are multiple forward paths from x_1 to x_j. Further, since the network is structurally controllable from x_1, there exists a break-away node (x_{ba}) from which two path diverges. Therefore, the dynamics of x_p and x_q can be expressed as two functions $g_p(x_{ba})$ and $g_q(x_{ba})$ respectively. Then the adaptation equation for the output node x_k can be written as

$$\dot{x}_k\bigg|_{\mathbf{x}^*} = 0 = \sum_{i=1}^{\mathfrak{K}(\mathcal{F}_k)} f_{\mathcal{F}_{ki}}(x_1(v), k_0) - f_{k,k}(k_0)$$

Where, $f_{\mathcal{F}_{ki}}$ is the contribution of the i^{th} forward path in $\mathcal{F}_k$ to the dynamics of x_k, k_0 is the constant steady-state concentration of x_k. Therefore, for a step change in the disturbance

$$\sum_{i=1}^{\mathfrak{k}(\mathcal{F}_K)} \Delta f_{\mathcal{F}_{ki}}(\Delta x_1(v), k_0) = 0 \tag{29}$$

(29) can only be satisfied if there exists at least a pair of elements in $\{f_{\mathcal{F}_{ki}}\}\ \forall\ i = 1(i)\mathfrak{K}(\mathcal{F}_k)$ has opposite signs. Let us consider without any loss of generality that contribution of $\mathcal{F}_p$ and $\mathcal{F}_q$– the paths consisting of nodes x_p and x_q respectively have opposing effects in the dynamics of x_k – This can only happen if the cumulative signs of these two paths after x_{ba} are opposite to each other, implying the presence of incoherency. □

Theorem 2 provides the vital requirements for perfect adaptation in a feed-forward network. As a next step, we shall investigate the scenario of loop networks wherein we shall attempt to provide a set of structural conditions that can be safely eliminated from the set of admissible network structures for perfect adaptation.

Theorem 3. *An N-node single-loop, controllable network without incoherent feed-forward paths can not provide perfect adaptation if the loop contains an edge from the output to the input-receiving node.*

Proof. Consider an N-node network $\mathcal{G}$ with a single loop (L_p) involving N_p number of nodes, including the input-receiving node. The controllability condition guarantees at least one path from x_1 to every other node in the network. Suppose, there exists an edge from any node $x_l \in N(\mathcal{G})\backslash N(L_p)$ (where $N(\mathcal{G})$ returns the node set of the network) to $x_p \in N(L_p)$. It is to note here since $x_p \in N(L_p)$, there exists a path from x_p to x_1 – This implies the existence of a loop other than L_p involving the forward path from x_1 to x_l and x_l to x_p and x_p to x_1 thereby violating the condition of a single loop.

Since there exists only one loop and no upstream connection from the non-loop nodes to the nodes of L_p, it is possible to show through constructive proof (Supporting information) that the resulting dynamics of the L_p can be expressed as

$$\dot{v} = 0 \tag{30}$$

$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \vdots \\ \dot{x}_k \vdots \end{bmatrix}_{\mathbf{x}=\mathbf{x}^*} = \begin{bmatrix} f_{11}(x_1) + f_{1v}(x_1, v) + f_{1k}(x_1, x_k) \\ f_{21}(x_2, x_1) + f_{22}(x_2) \\ \vdots \\ f_{kk-1}(x_k, x_{k-1}) + f_{kk}(x_k) \end{bmatrix}_{\mathbf{x}=\mathbf{x}^*} = \mathbf{0} \tag{31}$$

Putting the infinite precision condition $x_k^* = k_0$ on equation 31 and applying the assumption that f_{x_i,x_j} is a class-$\mathcal{K}$ function of x_j.

$$x_{k-1}^* = g(k_0) \implies x_1^* = h(k_0) = \text{ Constant} \tag{32}$$

It can be observed that for (31) and (32) to be consistent if and only if the external disturbance level v is kept constant, which defies the entire purpose of adaptation. Therefore this has to be treated as a contradiction. □

It is to be noted that, although Theorem 3 suggests the presence of only one loop, the main result works even if there exist multiple loops involving only those nodes that do not figure in L_p. Further, even in the case of multiple loops with an edge from the output to the input node, it can be shown that the network structure fails to provide perfect adaptation (Supporting information).

From Theorem 1, it is only appropriate to consider networks with at least N edges. Similarly, Theorem 3 also eliminates the set of network structures where all the loops include an edge from the output to the input-receiving node. Further, Theorem 2 excludes the coherent feed-forward networks from the set of admissible topologies for perfect adaptation. Therefore, we shall only investigate those network structures that pass the checkpoints proposed in Theorems 1, 2, and 3.

Remark 1. *As demonstrated in the foregoing sections, for an N-node network to provide perfect adaptation, it has to provide infinite precision (*(11) *and* (12)*) along with non-zero sensitivity (*(9)*). It is to be observed that* (11) *is a system of N equations. From our analysis of two and three-node networks, we have seen a particular equation in the system of equations in* (11) *that guarantees the constant steady-state value of the output state. We shall denote this equation as the invariance equation. Suppose there exists a particular node x_c in the network such that the following relation holds*

$$x_c \in \mathcal{F}_{ki} \forall i = 1(i)\mathfrak{k}(\mathcal{F}_k)$$

where, $\mathcal{F}_k$ contains all the forward paths from the input-receiving node to the output node. Further, x_c is the immediate node before x_p for every forward path in $\mathcal{F}_k$. In this scenario, it can be shown that x_p inherits the response of x_c in the context of adaptation, provided there is no loop between x_c and x_k. Therefore, the structural requirements that guarantee perfect adaptation for x_c act as a sufficient condition for the same for x_p.

Remark 2. *Further, the invariance equation can be situated either in the equation concerning the output node x_k (or x_c if it exists) or any other node. As the next step, we shall investigate both these two cases separately.*

In the first scenario, the dynamics of the output node or x_c (if it exists) can be written *as*

$$\dot{x}_k = \sum_{i=1}^{k} f_{x_k x_{Fi}}(x_k, x_{Fi}) - f_{k,k}(x_k)$$

where, k refers to the incoming degree of x_k and x_{Fi} is the immediate node before x_k for the forward path $\mathcal{F}_{ki}$.

According to the assumption, $f_{x_p,x_{Fi}}$ are class $\mathcal{K}$ function of x_{Fi}, the possibility of making it independent of x_{Fi} can be safely eliminated. Therefore, the only way the equation corresponding $\dot{x}_p$ can become the invariant equation is if, for a change from v_1 to $v + \Delta v$, the following condition holds true

$$\sum_{i=1}^{k} f_{x_p x_{Fi}}(x_{Fi}(v + \Delta v)) - \sum_{i=1}^{k} f_{x_p x_{Fi}}(x_{Fi}(v)) = 0 \tag{33}$$

It can be proved that due to the class $\mathcal{K}$ nature of $f_{i,j}(.)$ the steady state solution $\mathbf{x}^*(v)$ to (6) is a monotone function of v. Therefore, $\Delta v \geq 0 \implies |f_{x_p x_{Fi}}(x_{Fi}(v+\Delta v)) - f_{x_p x_{Fi}}(x_{Fi}(v)| > 0$. Therefore (33) can only be satisfied if at least one forward path exists whose effect on the dynamics of x_k is opposite to that of the rest of the forward paths. It is to be observed that none of the following conclusions change when x_c exists. In that case, instead of focusing on the equation concerning $\dot{x}_k$, the dynamics of x_c should be considered.

Remark 3. *In the second case, we examine the scenario where the invariance equation is neither the output state equation nor the one corresponding to x_c. We assume that the invariance equation is situated at the state equation of a node x_b ($x_b \neq x_c$, $x_b \neq x_k$). It is evident that since the invariance equation ensures the constant steady-state value of the output state x_k has a path from the output node x_k. Therefore, the dynamics equation for x_b can be written as*

$$\dot{x}_b = \sum_{i=1}^{k_b} f_{x_b x_{F_b i}}(x_b, x_{F_{bi}}) - f_{b,b}(x_b) \tag{34}$$

It is to be noted that at least one of $\{F_b i\}$ *has a path from the output node* x_k. *Considering the class* $\mathcal{K}$ *nature of* $f_{x_b, x_{F_{bi}}}$, *the only way* (34) *serves as the invariance equation at the steady state rendering* $x_k^* = k_0$ *is if* $\dot{x}_b$ *becomes independent of* x_b. *In this manner, the second term of* (34) *remains a constant, and the first term is independent of* x_b, *thereby making* $x_k = k_0$ *a possibility.*

Further, subsequent analysis of the local stability of the system reveals that the node x_b *has to engage in a negative feedback loop for stability (supporting information). The reason, intuitively, can be expressed in the following way. Since,* f_{x_b} *is independent of* x_b, *the corresponding* x_b^{th} *row in the system matrix* ($\mathbf{A}$) *of the linearised system has zero diagonal component. Therefore, from combinatorial matrix theory, the elements of the* ${x_b}^{textth}$ *row have to figure in the determinant expression within at least one loop expression.*[17]

3.4 Towards global stability

Remarks 1-3 establish the fact the structural recommendations obtained by Theorems 2, and 3 serve as necessary structural conditions for local, perfect adaptation in a network of any size. Since, by definition, global stability implies local (linearized) stability around the steady state, the structural conditions hitherto derived serve as the superset for the structures capable of global, perfect adaptation. Therefore, we begin the search for admissible network structures within the structural possibilities of incoherent feed-forward structure and negative feedback with buffer action.

It is to be noted that the states considered in the entire formalism refer to the concentration of the biochemical species (*eg.* genes or proteins). Therefore, the resulting dynamical systems underlying the biochemical networks are positive systems.

3.4.1 Global adaptation for IFF

Claim 1. *An incoherent feed-forward topology controllable by the input-receiving node with dynamics satisfying Assumptions (a)-(g) provides perfect adaptation for arbitrarily large in-*

put disturbance if each node has at least one incoming activation link.

Proof. Let us first define the biologically feasible range of the steady state $\Omega \subset \mathbb{R}^{N+} := \mathbf{x}_{\min} \leq \mathbf{x} \leq \mathbf{x}_{\max}$. We shall approach the proof in two steps: at first, we prove the uniqueness of the steady state in Ω and further prove the stability of that steady state to establish the stability of the steady state for any initial condition in Ω.

Uniqueness of the steady state : Without any generality loss, the input-receiving node's concentration is represented by the state x_1. Given a feed-forward structure (with no cycle) with the network being structurally controllable by the input-receiving node, the dynamics can be expressed as a triangular system

$$\dot{x}_1 = f_{1v}(x_1, v) - f_{11}(x_1) \tag{35}$$

$$\dot{x}_j = \sum_{k=1}^{j-1} f_{x_j,x_k} - f_{jj}(x_j) \tag{36}$$

where, the rate function $f_{ii}(x_i)$ refers to the self-degradation rate of each species. At the steady state,

$$0 = f_{1,v}(x_1, v) - f_{11}(x_1)$$

$$0 = \sum_{k=1}^{j-1} f_{x_j,x_k} - f_{jj}(x_j)$$

It is to be noted here that according to the statement of the claim, the rate function $f_{1,v}$ describes an activation reaction of x_1 by the disturbance input v. From Assumption f, for a given disturbance level v, $|f_{1,v}|$ and f_{11} are monotonically decreasing and increasing functions of x_1 respectively. Further, at a non-zero positive disturbance level, $f_{1,v}(x_1, v) > f_{11}(x_1) = 0\Big|_{x_1 = x_{1_{\min}}}$ and $f_{11}(x_1) > f_{1,v}(x_1, v) = 0\Big|_{x_1 = x_{1_{\max}}}$. This guarantees the existence of single, isolated steady-state x_1^* in the region $[x_{1_{\min}}, x_{1_{\max}}]$. Similarly, the other states also attain a unique, isolated solution at the steady state. This concludes the first part of the proof.

Stability : As it can be seen from Equations 35 and 36, at $x_i = x_{i_{\max}}$ $f_i < 0$, $\forall \mathbf{x} \in \Omega$

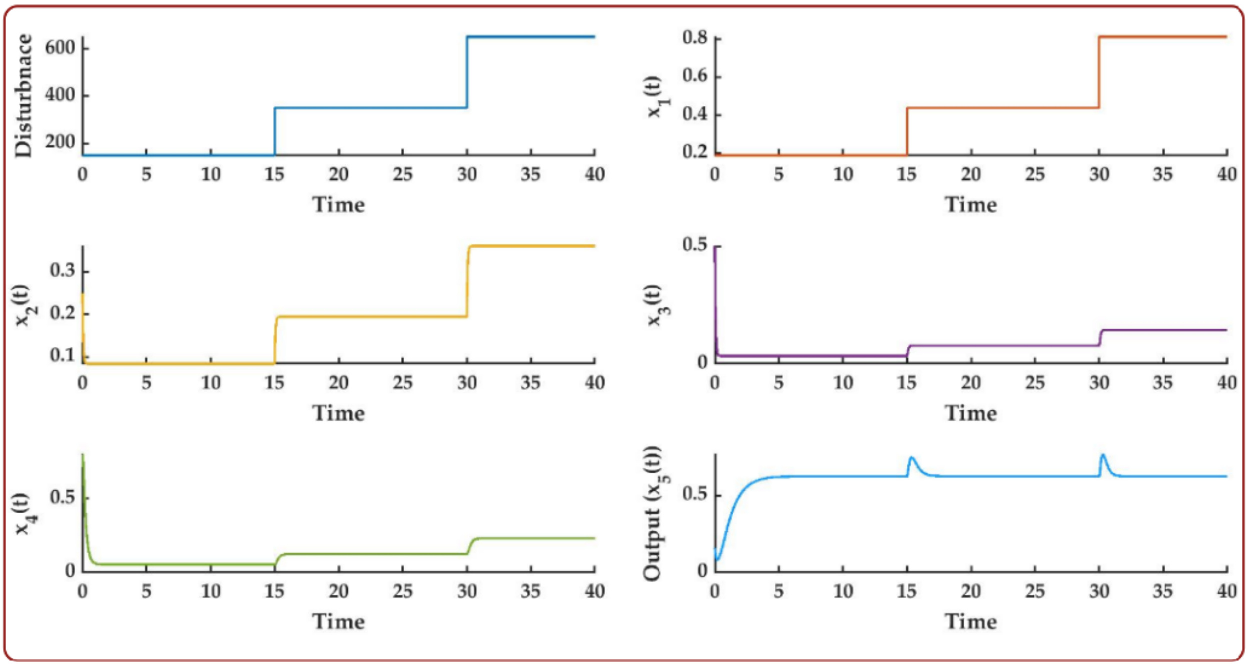

Figure 4: Encapsulates the conclusion drawn from Claim 1. Although the input disturbance is moved from an initial value of 0.3 to 600. The five-node, opposer module retains its adaptive property. Further, as depicted in the claim, all the states lie within $\Omega := \mathbf{0} \leq \mathbf{x} \leq \mathbf{1}$. The initial stiffness of the output state is due to the non-steady state initial condition.

implying $\Delta(x_i - x_{i_{\max}})\mathbf{f}(\mathbf{x}, v) < 0\Big|_{x_{i_{\max}}}$ due to the customary presence of self-degradation activities and optional repression at each node. Further, due to the presence of at least one activating effect present at each node, $x_i = x_{i_{\min}}\ f_i > 0.\ \forall \mathbf{x} \in \Omega, \implies \Delta(x_{i_{\min}} - x_i)\mathbf{f}(\mathbf{x}, v) < 0\Big|_{x_{i_{\min}}}$. Therefore, from Nagummo's theorem on positive invariance, we conclude that the compact set Ω is positive invariant in the current setting of feed-forward networks. Due to the feed-forward module's triangular structure, the system's Jacobian obtained around the only steady state $\mathbf{x}^* \in \Omega$ adopts a lower triangular structure. Due to the customary presence of the self-degradation and Assumptions a, f, and g, the diagonals of the Jacobian are always negative anywhere in Ω rendering the only steady state $\mathbf{x}^* \in \Omega$ locally stable. Ω is the biggest positive invariant set for the dynamical system containing a single singularity $\mathbf{x}^*$. Further, $\mathbf{x}^*$ is locally stable. Therefore, all the trajectories starting at Ω have to converge to $\mathbf{x}^*$ for the well-posed dynamical system, thereby making the system asymptotically stable in Ω. This concludes the proof. Fig. 4 demonstrates this result via a five-node opposer module in the presence of disturbance undergoing large amplitude swings. Fig. 5 lays out different feed-forward structures and elucidates this theoretical result. □

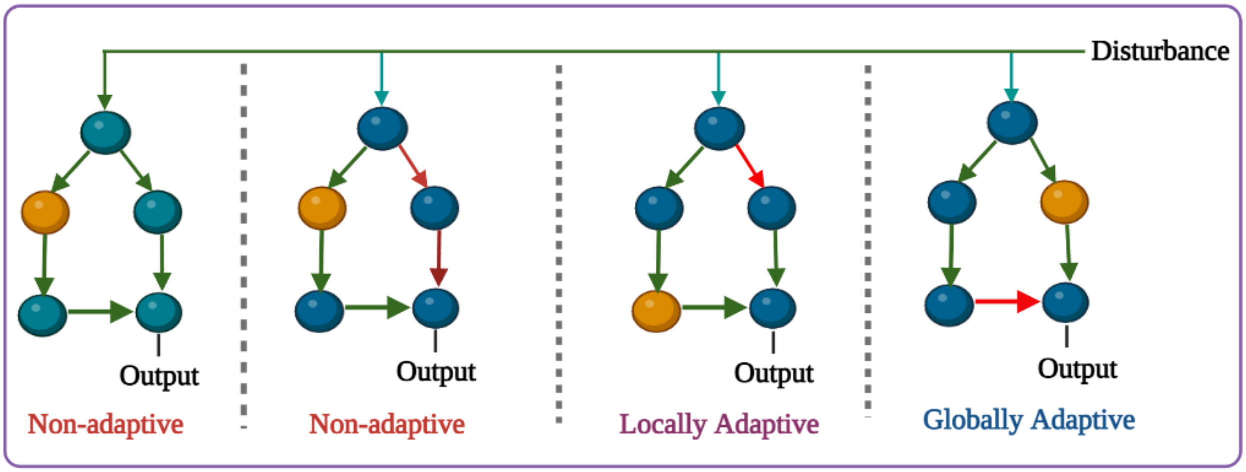

Figure 5: Different feed-forward structures relevant for perfect adaptation. The first two structures from the left fail to satisfy the opposing action, thereby failing to provide adaptation. On the other hand, the intermediate network structures, albeit satisfying the structural condition for local, perfect adaptation, can not guarantee a unique and single steady state, thereby failing to guarantee global properties. On the other hand, the right-most network structure guarantees a unique, stable, steady state in Ω, thereby exhibiting global adaptive properties. The node in Brown represents the controller node. The edges in Green and Red refer to activation and repression, respectively.

3.4.2 Global adaptation for NFB

Claim 2. *An $N-$node network structure containing a single feedback loop with an odd number of repressive edges and the underlying open-loop dynamics satisfying Assumptions (a)-(g) provides perfect adaptation for arbitrary large disturbance input if the following conditions are satisfied*

1. *Each node contains at least one incoming activation link.*

2. *The buffer control action is exerted directly on the input-receiving node.*

3. $|f_{kk}(x_K)| > |f_{k+1,K}(x_{k+1}, x_k)|$, $x_k > x_k^*$, $x_{k+1} > x_{k+1}^*$ *and*

   $|f_{kk}(x_K)| < |f_{k+1,K}(x_{k+1}, x_k)|$, $x_k < x_k^*$, $x_{k+1} < x_{k+1}^*$

Proof. Similar to IFF, we also attempt to establish the claim in two steps.

Uniqueness of the steady state : Without any loss of generality, i) let us denote the input-receiving node concentration as $x_1(t)$ and ii) assume the loop engages all the N nodes. Since the controller action is meditated through $x_1(t)$, the concentration of the controller node is indexed as $x_N(t)$. Therefore, the entire network can be understood as $x_1(t) \rightarrow x_2(t) \rightarrow x_2(t) \rightarrow \cdots \rightarrow x_N(t) \rightarrow x_1(t)$. The associated dynamics can be expressed as

$$\dot{v} = 0 \tag{37}$$

$$\dot{x}_1 = f_{1v}(x_1, v) + f_{1N}(x_1, x_N) - f_{11}(x_1) \tag{38}$$

$$\dot{x}_j = f_{j,j-1}(x_j, x_{j-1}) - f_{jj}(x_j) \tag{39}$$

$$\dot{x}_N = f_{N,N-1}(x_{N-1}) - k \tag{40}$$

It is established in Theorem 3 and Remark 3 that for negative feedback network structure, the controller dynamics have to be independent of its concentration. Therefore, equation (40) contains no term containing x_N in its right-hand side. At steady state, since $f_{N,N-1}$ is a monotonically increasing function of x_{N-1}, the solution to Equation (40) is unique. Further, due to the customary presence of at least one incoming, activating edge at each node, the unique solution x^*_{N-1} renders a unique steady state solution $\mathbf{x}^* \in \mathbb{R}^{N+}$ through the preceding equations. This satisfies the uniqueness criterion.

Stability : The dynamical system in Equations (37) – (40) assumes a triangular form with respect to $(v, \mathbf{x})$. From Vidyasagar's theorem on triangular system,[30] the Lyapunov stability of the interconnected system can be established if we can establish the asymptotic stability of the autonomous dynamical system described in Equations (38) – (40). For this purpose, we propose the following Lyapunov functional

$$V(x) = \sum_{k=1}^{N} ||\tilde{\mathbf{x}}||_1 \tag{41}$$

where, $\tilde{\mathbf{x}} := \mathbf{x} - \mathbf{x}^*$. Further, since there exists a state-dependent degradation at every node from x_2 to x_{N-1}, the corresponding contribution of the dynamics $(f_2 - f_{N-1})$ to the time derivative $\dot{V}$ contains at least one negative sign. Further, in the special scenario of $\tilde{\mathbf{x}} > 0$ and $\tilde{\mathbf{x}} < 0$, the contribution of f_N to $\dot{V}$ is positive. However, since f_{1N} is a repressing edge, the positive contribution of f_N is nullified by the same of f_1 rendering the overall expression $\dot{V}$ negative (note that x_N^* in the case of an autonomous system is, unique but negative) owing to the third condition in the statement of the claim. This proves the Lyapunov stability of the interconnected system. Fig. 7 provides the schematic of different possible adaptive balancer modules. A simulation study for a balancer module in the presence of large disturbances is demonstrated in Fig. 6. $\square$

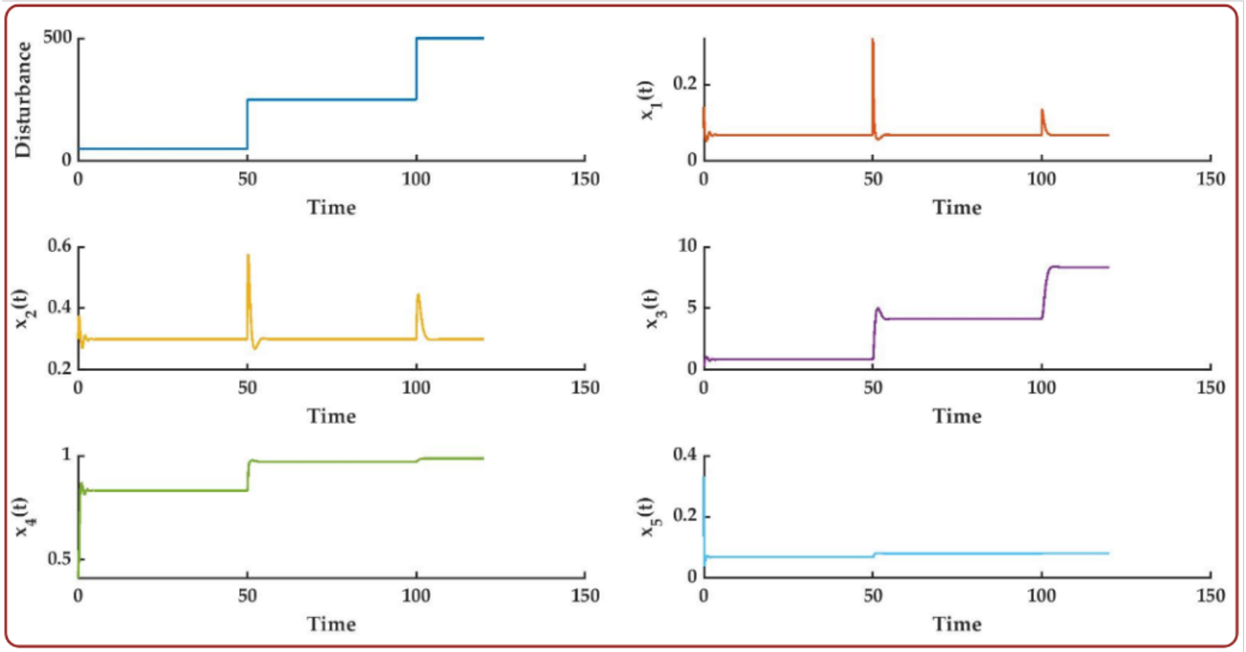

Figure 6: Encapsulates the conclusion drawn from Claim 2. Although the input disturbance is moved from an initial value of 0.3 to 600. The five-node balancer module retains its adaptive property. Further, as depicted in the claim, all the states lie within $\Omega := \mathbf{0} \leq \mathbf{x} \leq \mathbf{1}$ except the controller node. It can be shown that, unlike the opposer modules, the concentration of the controller node, the node that accomplishes the buffering action, can not be contained within $[0, 1]$.

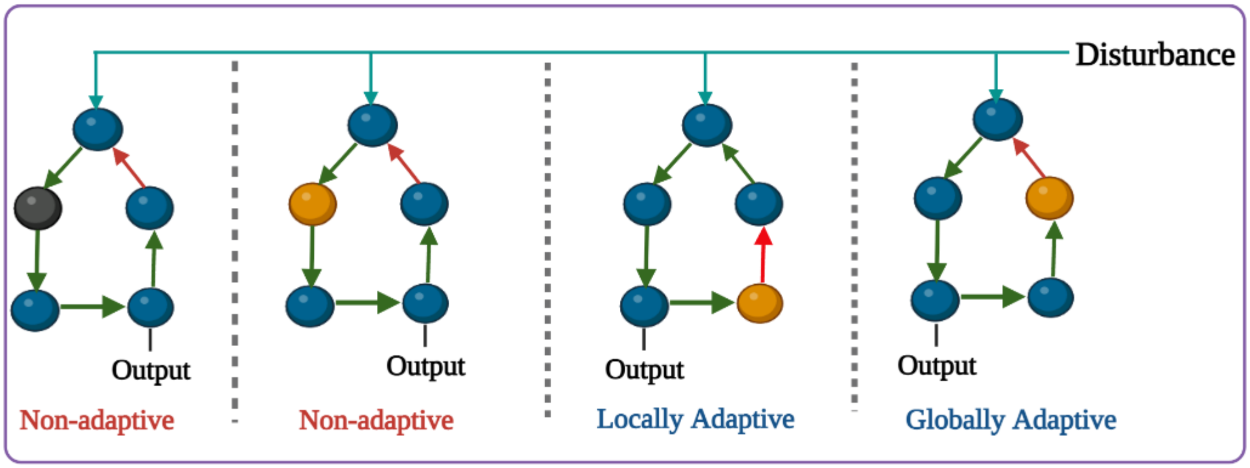

Figure 7: Different feed-forward structures relevant for perfect adaptation. The first two structures from the left fail to satisfy the buffer action, thereby failing to provide adaptation. On the other hand, the intermediate network structures, albeit satisfying the structural condition for local, perfect adaptation, can not guarantee a unique and single steady state, thereby failing to guarantee global properties. On the other hand, the right-most network structure guarantees a unique, stable, steady state in the state space, thereby capable of exhibiting global adaptive properties. The node in Brown represents the controller node providing the buffer action crucial for adaptation. The edges in Green and Red refer to activation and repression, respectively.

Remark 4. *It can be seen that due to the buffer action by the controller node and the condition (b) of Claim 2, all the $N-1$ nodes of the loop (excluding the controller node) performs perfect adaptation. In general, for any loop engaging P number of nodes, $P-1$ nodes can perform perfect adaptation if and only if the controller node exerts the control action through the input-receiving node. We can further relax the first requirement of an edge from the controller to the input node as the first $K-1$ nodes starting from the input-receiving node to the one before the controller node (the K^{th} node) can perform perfect adaptation.*

3.5 Global adaptation in the presence of downstream connection

Most of the analysis in the foregoing sections assumes that the network structure is isolated from any downstream network, which is not the case in reality. Typically, adaptation networks are mounted on top of big biological networks to improve the cell's robustness against variations in the external environment. It is, therefore, necessary to investigate the performance of an adaptive network in the presence of downstream connections. For this purpose, we adopt the standard model of downstream connection known as retroactivity developed by.[31,32] In this model, the output node of the adaptive network is connected cyclically with any of the downstream nodes. From Remark 3, it is well-known that the invariance equation is situated at the controller node in the case of negative feedback. Therefore, the steady-state value of the output node is decided at the steady-state equation pertaining to the controller node itself. Therefore, the steady-state value of the output node is preserved in the presence of a downstream connection. To expand this further, the dynamical system underlying an NFB network (in Equations (38) – (40)) can be expressed at steady as

$$f_{N,N-1}(x^*_{N-1}) - k = 0 \tag{42}$$

$$\implies x^*_{N-1} = k_1 \tag{43}$$

$$f_{N-1,N-2}(x^*_{N-1}, x^*_{N-2}) - f_{N-1}(x^*_{N-1}) = 0 \tag{44}$$

$$\implies x^*_{N-2} = k_2 \tag{45}$$

$$\tag{46}$$

Suppose the output node is the K^{th} node. The $(K+1)^{\text{th}}$ steady state equation can be written as

$$f_{K+1,K}(x^*_{K+1}, x^*_K) - f_{K+1}(x^*_{K+1}) = 0 \tag{47}$$

$$\implies x^*_K = \tilde{k} \quad (x^*_{K+1} \text{ is constant due to previous equation.}) \tag{48}$$

Therefore, if the stability of the network structure is not altered, the perfect adaptation property of the output node of a negative feedback network with a buffer node is conserved in the presence of a downstream network. Fig. 8 illustrates the modular nature of balancer modules with a simulation exercise.

On the other hand, the incoherent feed-forward topology, as established in Remark 2, achieves perfect adaptation through the invariance equation situated at the output node (or to another node x_c in the network given the output node contains nothing other than a single incoming path from node x_c). Therefore, the invariance equation is modified if the output node is connected to the downstream network. Let us consider an adaptation-capable, feed-forward network with a downstream node x_d and the output being the concentration of $(j+1)^{\text{th}}$ node.

$$\dot{v} = 0 \tag{49}$$

$$\dot{x}_1 = f_{1,v}(x_1, v) - f_{1,1}(x_1) \tag{50}$$

$$\dot{x}_j = \sum_{k=1}^{j-1} f_{j,k}(x_j, x_k) - f_{j,j}(x_j) \tag{51}$$

$$\dot{x}_{j+1} = \sum_{k=1}^{j} f_{j+1,k}(x_j, x_k) - f_{j+1,j+1}(x_{j+1}) \pm f_{j+1,d}(x_{j+1}, x_d) \tag{52}$$

The condition for perfect adaptation in the isolated scenario is

$$\Delta \sum_{k=1}^{j} f_{j+1,k}(x_j, x_k) = 0$$

where ‘Δ’ refers to the change in the functional values due to the change in the disturbance level v. Further, the condition for the same in the presence of the downstream connection can be written as

$$\Delta f_{j+1,d}(x_{j+1}, x_d) = 0 \tag{53}$$

Equation (53) puts the baggage of adapting to the downstream state x_d, showcasing the inability of feed-forward networks to provide global, perfect adaptation in the presence of downstream connection. However, Equation (53) can be satisfied if the steady state of x_d is kept to zero. In that case, due to the introduction of negative feedback in the otherwise feed-forward structure, the corresponding response for x_d shall be oscillatory, leading to a 'negative' value of concentration. One can find a strong parallel to this theoretical conclusion with the computational work by[33] in their study of how incoherent feed-forward loops can perform perfect adaptation (maintain constant output levels) for a specific range of retroactive strength. Fig. 8 vividly illustrates the retroactive nature of the opposer modules with a simulation exercise.

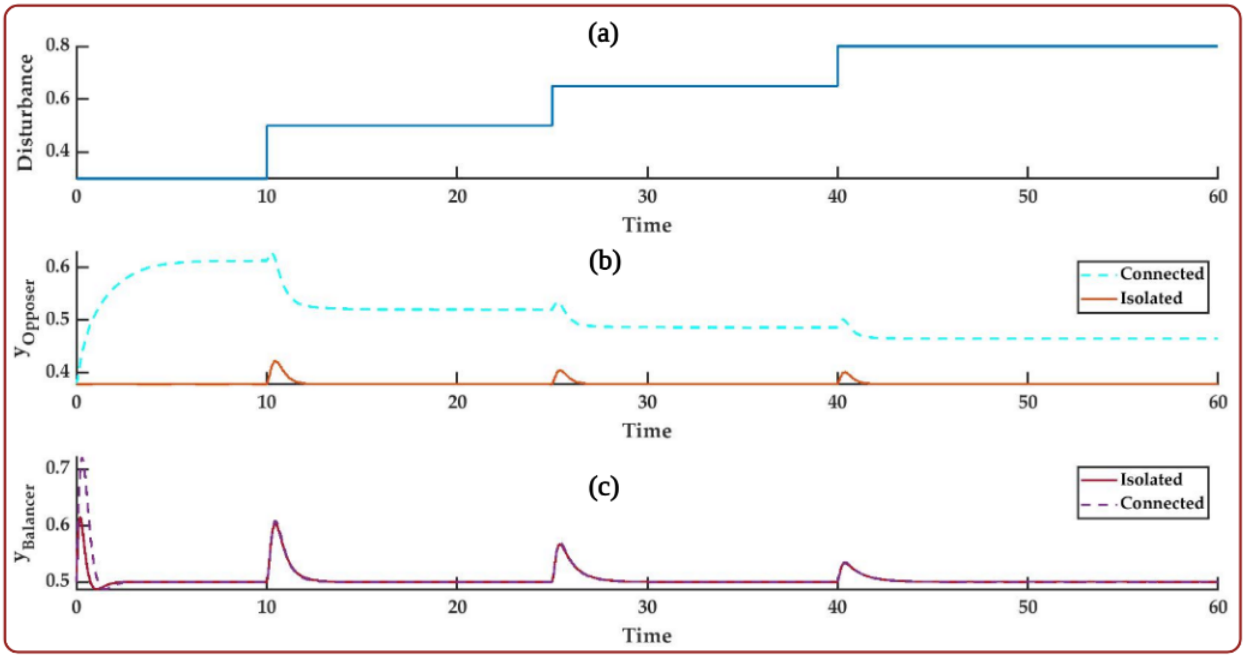

Figure 8: A comparative study of the retroactivity property of the balancer and the opposer modules. As it can be seen in (b) that a perfectly adaptive, 3−node opposer module loses its adaptation capability when connected to a downstream system consisting of two nodes. On the other hand, as shown in (c), a 3−node balancer module retains its adaptation capability even in the presence of a two-node downstream connection. Interestingly, the output response remains the same irrespective of the manner of the connections (positive or negative loops) between the output node and the downstream system.

4 Discussion

The inherent nonlinearity and variety of possible rate dynamics contribute a significant share to the complexity of the biological networks. Apart from the apparent abundance of rate dynamics, it is well-established in the literature[1,34] that the network structure plays a governing role in determining the response of a network structure. The present study follows from this seminal observation and attempts to synthesize a methodology that provides novel structural insights without possessing any explicit dependence on the particular rate kinetics.

The existing literature, including[1,4,5] has focused chiefly on the computational approaches to deduce the design principles for perfect adaptation despite the obvious scalability issue. Further, rule-based approaches such as[7,8] proposes a negative feedback-based structure that can provide perfect adaptation. Apart from these two approaches, other theoretical interventions primarily rest on the treatment of the dynamical system in the linearised domain[13,14,16,17] – This can not portray the global picture, albeit being a sophisticated approach, – nor can it incorporate realistic constraints that can be thought of as the vital design criteria from the vantage point of the synthetic design. To circumvent this problem, the current chapter proposed a methodology inspired by nonlinear systems theory that strives to draw novel structural insights about biological networks that can produce perfect adaptation in a global sense. At first, we determined the conditions for perfect adaptation using the classical performance parameters for adaptation, namely sensitivity, and precision. Contrary to a Jacobian-based approach prevalent in the existing systems-theoretic approaches, we proposed the condition for infinite precision as the existence of an error-zeroing manifold in the state space. On the other hand, the sensitivity condition is met through a Lie-controllability test of the dynamical system. At this point, global adaptation is indeed a stronger requirement than an infinitesimal adaptation for the added conditions of strict global stability and a unique steady state.

The proposed framework has been used to study the biochemical networks. To verify the righteousness of the algorithm, we first applied this to deduce the optimal network structures for perfect adaptation in a localized sense. Evidently, the structural predictions obtained are identical to the ones obtained through the Jacobian treatment of the linearised system. Additionally, the proposed methodology can also deal with the case of singular Jacobians using the principle of Central Manifold Theory. In that case, instead of the concentration of a particular node, the linear combination of the node concentrations is likely to be able to produce an adaptive response.

The network structures obtained for local adaptation serve as the superset for the structures capable of global, perfect adaptation. Propositions 1, 2, and 3 provide the necessary structural requirements for local, perfect adaptation in networks of small size. Subsequently, the scope of these results is generalized using Theorems 1, 2, and 3 to establish that negative feedback with buffer node or multiple feed-forward structures with mutually opposite effects on the output node is the key to local, perfect adaptation in a network of any size.

Intuitively, the structural requirements for local, perfect adaptation can only serve as the necessary conditions for global adaptive characteristics. Claim 1 establishes that '*not all incoherent feed-forward network can provide global adaptation.*' Only those feed-forward structures wherein each node contains at least one activating incoming edge can provide global (in Ω), perfect adaptation. Similarly, Claim 2 also produces such a subclass of the networks containing a negative feedback loop with buffer action in the context of global, perfect adaptation. It is to be noted at this juncture that the rate law assumptions (Assumptions (f), (g)) are only used in proving the global stability of the steady state. The monotone nature of the rate functions (standard across the existing rate laws) is sufficient to guarantee the uniqueness of the steady state. The curious case of synergistic or mass-action rate laws has already been well-discussed by[27,28] through the construction of a particular Lyapunov function obtained from linear programming. Therefore, the analysis with Assumptions (g) and (f) brings completeness to the study of the global stability of biological networks.

Finally, contrary to the conclusions drawn through the linear analysis, the proposed methodology reveals the inability of the feed-forward loop to retain the global adaptive property. The linearised analysis leads to a system of linear algebraic equations with a trivial solution for the (linearised) downstream system. In contrast, due to the nonlinearity of the rate functions, the actual steady-state solutions for the downstream states are likely to be non-zero and dependent on the disturbance input, thereby resulting in the violation of the adaptation property of the upstream feed-forward module.

Moreover, the linearised or local analysis of the system reveals the necessary paths (with its sign) and loops for perfect adaptation. In contrast, the practical constraints and global stability conditions allow us to zoom in further to obtain the appropriate edge configurations out of all the candidate loops and paths capable of local, perfect adaptation. Subsequently, investigating the robustness of each network structure admissible for global adaptation and obtaining appropriate structural prediction for robust, perfect, and global adaptation can be an exciting area of further study.

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