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Infinitesimal homeostasis in three-node input–output networks

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Abstract

Homeostasis occurs in a system where an output variable is approximately constant on an interval on variation of an input variable $\mathcal{I}$. Homeostasis plays an important role in the regulation of biological systems, cf. Ferrell (Cell Syst 2:62–67, 2016), Tang and McMillen (J Theor Biol 408:274–289, 2016), Nijhout et al. (BMC Biol 13:79, 2015), and Nijhout et al. (Wiley Interdiscip Rev Syst Biol Med 11:e1440, 2018). A method for finding homeostasis in mathematical models is given in the control theory literature as points where the derivative of the output variable with respect to $\mathcal{I}$ is identically zero. Such points are called *perfect homeostasis* or *perfect adaptation*. Alternatively, Golubitsky and Stewart (J Math Biol 74:387–407, 2017) use an infinitesimal notion of homeostasis (namely, the derivative of the input–output function is zero at an isolated point) to introduce singularity theory into the study of homeostasis. Reed et al. (Bull Math Biol 79(9):1–24, 2017) give two examples of infinitesimal homeostasis in three-node chemical reaction systems: feedforward excitation and substrate inhibition. In this paper we show that there are 13 different three-node networks leading to 78 three-node input–output network configurations, under the assumption that there is one input node, one output node, and they are distinct. The different configurations are based on which node is the input node and which node is the output node. We show nonetheless that there are only three basic mechanisms for three-node input–output networks that lead to infinitesimal homeostasis and we call them *structural homeostasis*, *Haldane homeostasis*, and *null-degradation homeostasis*. Substantial parts of this classification are given in Ma et al. (Cell 138:760–773, 2009) and Ferrell (2016) among others. Our contributions include giving a complete classification using general admissible systems (Golubitsky and Stewart in Bull Am Math Soc 43:305–364, 2006) rather than specific biochemical models, relating the types of infinitesimal homeostasis to the graph theoretic existence of simple paths, and providing the basis to use singularity theory to study higher codimension homeostasis singularities such as the chair singularities introduced in Nijhout and Reed (Integr Comp Biol 54(2):264–275, 2014. https://doi.org/10.1093/icb/icu010) and Nijhout et al. (Math Biosci 257:104–110, 2014). See Golubitsky and Stewart (2017). The first two of these mechanisms

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are illustrated by feedforward excitation and substrate inhibition. Structural homeostasis occurs only when the network has a feedforward loop as a subnetwork; that is, when there are two distinct simple paths connecting the input node to the output node. Moreover, when the network is just the feedforward loop motif itself, one of the paths must be excitatory and one inhibitory to support infinitesimal homeostasis. Haldane homeostasis occurs when there is a single simple path from the input node to the output node and then only when one of the couplings along this path has strength 0. Null-degradation homeostasis is illustrated by a biochemical example from Ma et al. (2009); this kind of homeostasis can occur only when the degradation constant of the third node is 0. The paper ends with an analysis of Haldane homeostasis infinitesimal chair singularities.

Keywords Homeostasis · Networks · Singularity theory · Biochemistry

Mathematics Subject Classification 34C99 · 92C42 · 92C40

1 Introduction and results

Homeostasis refers to a phenomenon whereby the output x_o of a system is approximately constant on variation of an input $\mathcal{I}$. The terms perfect homeostasis and robust homeostasis are also used in the literature. According to Tang and McMillen (2016) *perfect homeostasis* means that the output is identically constant on a neighborhood of an input value $\mathcal{I}_0$ and *robust homeostasis* means that the system maintains homeostatic behavior when its parameter values are slightly altered. These authors note that the terms *adaptation* and *perfect adaptation* are used in the literature when referring to homeostasis in sensory systems. See also Ang and McMillen (2013), Aoki et al. (2019), and Qian and Del Vecchio (2018).

Many researchers have observed that homeostasis can be defined locally near an input value $\mathcal{I}_0$; in particular, this follows when the input–output function $x_o(\mathcal{I})$ is approximately constant near $\mathcal{I}_0$. Golubitsky and Stewart (2017) and Reed et al. (2017) call this a *homeostasis point*. We follow these authors by observing that homeostasis points occur when the derivative of x_o with respect to $\mathcal{I}$ is zero at $\mathcal{I}_0$. More precisely:

Definition 1 *Infinitesimal homeostasis* occurs at an isolated point $\mathcal{I}_0$ if

$$x_o'(\mathcal{I}_0) = 0$$

where $'$ indicates differentiation with respect to $\mathcal{I}$.

It follows from Taylor's theorem that infinitesimal homeostasis implies local homeostasis, though the converse need not be valid. See Reed et al. (2017) and the discussion of product inhibition in Sect. 2. Observe that perfect homeostasis is a much stronger condition since it requires that the output x_o be identically constant—not just that the derivative of x_o is zero at a point.

Homeostasis and infinitesimal homeostasis occur naturally in biochemical network models. We point to Nijhout et al. (2004), Ma et al. (2009), Nijhout and Reed (2014), Ferrell (2016), Tang and McMillen (2016), Del Vecchio et al. (2018), Reed et al. (2017), Antoneli et al. (2018) and references therein.

In the remainder of this section, we define input–output functions for networks, coupling types, state the main results, and outline the paper. Our results are motivated by the mathematics of singularity theory and the strength of these results depend on using a mathematically precise definition of homeostasis (namely, infinitesimal homeostasis) that was introduced in Golubitsky and Stewart (2017).

1.1 The input–output function in three-node input–output networks

This paper focusses on infinitesimal homeostasis in three-node input–output networks. We assume that one node ι is the *input node*, a second node o is the *output node*, and the third node ρ is the *regulatory node*. The network coordinates are $X = (x_\iota, x_\rho, x_o)$. We assume that the network system has the form $F = (f_\iota, f_\rho, f_o)$ where each coordinate function f_ℓ depends on the internal state variable x_ℓ and the state variables of the nodes coupled to node ℓ in the network graph.

We make three assumptions about the vector field F throughout:

(a) The phase space of each node as well as the input variable is one-dimensional; that is, $(x_\iota, x_\rho, x_o) \in \mathbf{R}^3$ and $\mathcal{I} \in \mathbf{R}$.
(b) Only the input node coordinate function f_ι depends on the external input variable $\mathcal{I}$ and the partial derivative of f_ι with respect to $\mathcal{I}$ is nowhere 0.
(c) F has a stable equilibrium at $(X_0, \mathcal{I}_0)$.

The definition of an input–output function begins with assumption (c) that

$$\dot{X} = F(X, \mathcal{I}) \tag{1.1}$$

has a stable equilibrium X_0 at $\mathcal{I}_0$. Stability and the implicit function theorem together imply that there exists an implicitly defined mapping $X(\mathcal{I})$ such that

$$F(X(\mathcal{I}), \mathcal{I}) \equiv 0 \tag{1.2}$$

where $X(\mathcal{I}_0) = X_0$. In coordinates $X(\mathcal{I}) = (x_\iota(\mathcal{I}), x_\rho(\mathcal{I}), x_o(\mathcal{I}))$, where $x_o(\mathcal{I})$ is the *input–output function*. In general, we can write the equilibrium equations of (1.1) as

$$\begin{aligned} f_\iota(x_\iota, x_\rho, x_o, \mathcal{I}) &= 0 \\ f_\rho(x_\iota, x_\rho, x_o) \quad &= 0 \\ f_o(x_\iota, x_\rho, x_o) \quad &= 0 \end{aligned} \tag{1.3}$$

Given a three-node network, the associated class of differential equations will always be a subclass of (1.3). For example, the feedforward motif illustrated in Fig. 1 has the corresponding systems of equations in (1.4).

$$\begin{aligned} \dot{x}_\iota &= f_\iota(x_\iota, \mathcal{I}) \\ \dot{x}_\rho &= f_\rho(x_\iota, x_\rho) \\ \dot{x}_o &= f_o(x_\iota, x_\rho, x_o) \end{aligned} \tag{1.4}$$

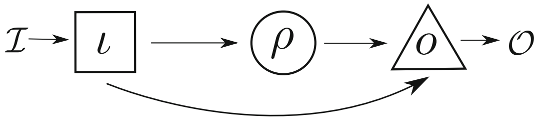

Fig. 1 The feedforward motif

Following Golubitsky and Stewart (2006) we call such equations *admissible systems* for the given network. Substantial parts of this classification are given in Ma et al. (2009) and Ferrell (2016) and other references. Our study of infinitesimal homeostasis differs from those studies of homeostasis in three ways. First, we classify infinitesimal homeostasis using general admissible systems rather than analyzing specific model systems, though our results could be used to analyze such systems. Second, we relate infinitesimal homeostasis to combinatorial facets of the network. Third, we take a singularity theory point of view (see Golubitsky and Stewart 2017), which provides a way to study higher codimension infinitesimal homeostasis singularities (see Sect. 4). The codimension of a homeostasis singularity is the minimal number of parameters that determine it, assuming suitable nondegeneracy conditions. These conditions lead to robust infinitesimal homeostasis.

Linearized coupling types

We use the notation $f_{\ell,y}$ to denote the partial derivative of the coordinate function f_ℓ with respect to y. Specifically, f_{ℓ,x_ℓ} denotes the linearized internal dynamics of node ℓ.

Definition 2 Suppose, in a given network, one node m connects to another node ℓ. Then the coupling is *excitatory* at the equilibrium X_0 if $f_{\ell,x_m}(X_0) > 0$, *inhibitory* if $f_{\ell,x_m}(X_0) < 0$, and *neutral* if $f_{\ell,x_m}(X_0) = 0$. Node ℓ *degrades* at the equilibrium X_0 if $f_{\ell,x_\ell}(X_0) < 0$.

Remark 3 Neutral coupling between two nodes does not mean that the nodes are uncoupled–rather it means that the coupling exists and is at a transition state between excitation and inhibition. Therefore, neutral coupling between two nodes is possible only when coupling between those two nodes is assumed possible. In many biochemical networks, but not all, degradation in each node is expected. In some networks, such as the feedforward motif (see (1.4)), stability of the equilibrium forces degradation.

1.2 Results on infinitesimal homeostasis

We ask the following question: Given a three-node network with designated input and output nodes, what conditions on the linearized couplings lead to the possible existence of infinitesimal homeostasis? The answer depends on the network structure–specifically the number of simple paths from the input node to the output node. We prove that even though there are 78 different input–output networks (see Appendix 6) there are precisely three different kinds of infinitesimal homeostasis (see Theorem 10 and Theorem 12). By a *kind of infinitesimal homeostasis* we mean that the vanishing of one specific combination of partial derivatives of f_ι, f_ρ, f_o, (a *defining condition*), leads to infinitesimal homeostasis given that the nondegeneracy condition $x_o''(\mathcal{I}_0) \neq 0$ holds. Moreover, we show that these three different types depend only on the graph theoretic simple path structure of the network.

Definition 4 A path between nodes is *simple* if the path visits each node on the path at most once.[1]

A three-node input–output network has three possible configurations of simple paths from input node to output node: $\iota \to o$ or $\iota \to \rho \to o$ or both. We show that a network containing both simple paths can lead to a kind of homeostasis that we call *structural*. A network containing the one simple path $\iota \to \rho \to o$ can lead to a kind of homeostasis that we call Haldane. Finally, a network containing the one simple path $\iota \to o$ can lead to *Haldane* homeostasis or to a kind of homeostasis that we call *null-degradation* homeostasis. More precisely, we prove the following.

(1) *Suppose the network has one simple path $\iota \to o$. It follows that either $\iota \not\to \rho$ or $\rho \not\to o$.* In this case, Theorem 10(a) shows that infinitesimal homeostasis occurs at $\mathcal{I} = \mathcal{I}_0$ if and only if either

   (a) (Haldane homeostasis) the linearized coupling along the path is neutral at $\mathcal{I} = \mathcal{I}_0$; that is,

$$f_{o,x_\iota}(X_0) = 0 \tag{1.5}$$

   or

   (b) (null-degradation homeostasis) the linearized internal dynamics of the regulatory node ρ is zero at $\mathcal{I} = \mathcal{I}_0$; that is,

$$f_{\rho,x_\rho}(X_0) = 0. \tag{1.6}$$

(2) *Suppose the network has one simple path $\iota \to \rho \to o$. It follows that $\iota \not\to o$.* In this case Theorem 10(b) shows that infinitesimal homeostasis (specifically Haldane homeostasis) occurs if and only if either the linearized coupling $\iota \to \rho$ is neutral or the linearized coupling $\rho \to o$ is neutral. That is, either

$$(a)\ f_{\rho,x_\iota}(X_0) = 0 \qquad \text{or} \qquad (b)\ f_{o,x_\rho}(X_0) = 0. \tag{1.7}$$

   at $\mathcal{I} = \mathcal{I}_0$.

(3) *The network has two simple paths from the input node ι to the output node o; namely, $\iota \to o$* and $\iota \to \rho \to o$. Theorem 12 shows that infinitesimal homeostasis can occur only when the coupling along the two paths from ι to o balance. That is, the system satisfies the defining condition (structural homeostasis)

$$f_{o,x_\rho}(X_0) f_{\rho,x_\iota}(X_0) - f_{o,x_\iota}(X_0) f_{\rho,x_\rho}(X_0) = 0 \tag{1.8}$$

   at $\mathcal{I} = \mathcal{I}_0$.

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[1] See http://www.people.vcu.edu/~gasmerom/MAT131/graphs.html.

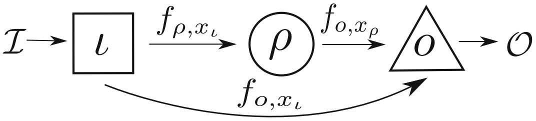

Fig. 2 Excitatory/inhibitory coupling in input–output feedforward loop motif

In the feedforward loop motif, stability of the equilibrium X_0 implies $f_{\rho,x_\rho}(X_0) < 0$ and the existence of the structural homeostasis is only possible if

$$f_{o,x_\iota}(f_{o,x_\rho} f_{\rho,x_\iota}) < 0 \tag{1.9}$$

at X_0; that is, one simple path is excitatory and the other inhibitory. Figure 2 illustrates (1.9). In general, in biochemical network modeling, the assumption that the ρ node degrades is reasonable and (1.9) is still valid.

Definition 5 (a) We call homeostasis of types (1a) and (2) *Haldane homeostasis*, since these types arise from neutral coupling and, in biochemical networks, require homeostasis be built into the kinetics function. Cf. Haldane (1930).
(b) We call homeostasis of type (1b) *null-degradation homeostasis*, since this type arises when the degradation constant (i.e., the linearized internal dynamics) of the regulatory node is zero.
(c) We call homeostasis of type (3) *structural homeostasis*,[2] since this type typically arises from non-neutral couplings and requires a balance of coupling strengths between two simple paths in the network.

Examples of Haldane homeostasis satisfying the definining condition (1.5), (1.7)a or (1.7)b are product inhibition (Sect. 2.3) and substrate inhibition (Sect. 2.2). A biochemical example of null-degradation homeostasis that satisfies the defining condition (1.6) is the negative feedback loop given by (Ma et al. 2009, Fig. 7). See Sect. 2.4. A biochemical example of the feedforward loop for structural homeostasis can be found in (Ferrell 2016, Fig. 3). Another example of structural homeostasis is feedforward excitation. See Sect. 2.1. Specifically, structural homeostasis satisfies the defining condition (1.8).

The three possible simple path configurations lead to equations for infinitesimal homeostasis that the one parameter $\mathcal{I}$ must satisfy at some point $\mathcal{I}_0$, that is, the infinitesimal homeostasis defining conditions. Consequently, infinitesimal homeostasis is a generic phenomenon in each input–output network; which form of infinitesimal homeostasis can occur depends on which simple paths are present in the network. From a singularity theory point of view infinitesimal homeostasis is generically (under the nondegeneracy condition $x_o''(\mathcal{I}_0) \neq 0$) of codimension 0. There is one obstacle. There are networks that cannot satisfy the infinitesimal homeostasis defining conditions at a *stable equilibrium.*

Remark 6 It follows from the classification of three-node networks (see the Appendix 6) that there are eight three-node input–output networks that support two simple paths

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[2] We thank Mike Reed for suggesting the term structural homeostasis.

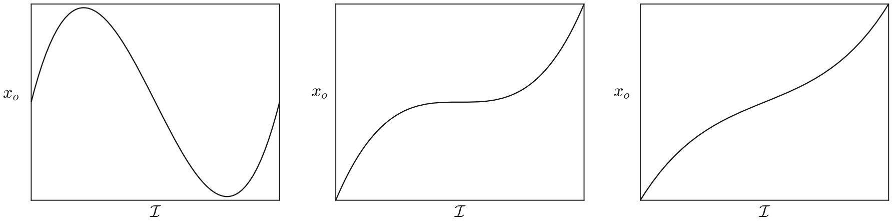

Fig. 3 Graphs of the input–output function are given as the universal unfolding parameter a varies in the chair singularity near a_0, see Golubitsky and Stewart (2017). From left to right: $a < a_0$, $a = a_0$ and $a > a_0$

from the input node to the output node. These networks include Fig. 10b, the feedforward loop motif, as a subnetwork, and they are pictured in Fig. 8. Theorem 12 shows that structural homeostasis (homeostasis with non-neutral coupling) can only occur in eight of the 78 possible three-node input–output networks.

1.3 Chairs in Haldane homeostasis

A consequence of the theorems discussed previously is that infinitesimal homeostasis occurs when $x_o'(\mathcal{I}_0) = 0$. Moreover, typical infinitesimal homeostasis occurs when in addition $x_o''(\mathcal{I}_0) \neq 0$. Elementary singularity theory then implies that $x_o(\mathcal{I})$ is shaped like a parabola on a neighborhood of the infinitesimal homeostasis point $\mathcal{I}_0$. See Golubitsky and Stewart (2017).

Nijhout and Reed (2014) and Nijhout et al. (2014) introduce homeostasis and its applications through *chairs* and the notion of *escape from homeostasis*. The basic observation is that as the input $\mathcal{I}$ varies the output $x_o(\mathcal{I})$ goes through three regions: increase, approximately constant, increase (see Fig. 3, middle panel) or decrease, approximately constant, decrease. Golubitsky and Stewart (2017) discuss an infinitesimal version of chairs that brings singularity theory into the study of homeostasis. An *infinitesimal chair* occurs at a point $\mathcal{I}_0$ if $x_o'(\mathcal{I}_0) = x_o''(\mathcal{I}_0) = 0$ and $x_o'''(\mathcal{I}_0) \neq 0$; that is, up to a change of coordinates the input–output function is a homogeneous cubic on a neighborhood of $\mathcal{I}_0$.

From a singularity theory point of view, a chair is a codimension one singularity. Near a chair singularity, the graph of x_o changes on variation of a single additional system parameter a, as illustrated in Fig. 3. Reed et al. (2017) showed that nonlinear degeneracies in substrate inhibition and feedforward excitation lead to infinitesimal chairs. We describe the precise equations describing chair existence in Haldane homeostasis in Sect. 4.

1.4 Organization of paper

Section 2 uses the theory stated in Sect. 1.2 to reproduce results in Reed et al. (2017) and Ma et al. (2009) that show that infinitesimal homeostasis can occur in three-node input–output networks with either non-neutral coupling (specifically in feedforward excitation) or with neutral coupling (specifically in substrate inhibition). For a detailed discussion of substrate inhibition see Reed et al. (2010). We also discuss the relationship between homeostasis and infinitesimal homeostasis in product inhibition.

The results stated in Sect. 1.2 are proved in Sect. 3. Haldane homeostasis chairs are discussed in Sect. 4 with proofs given in an Appendix 7. The classification of three-node networks is given in an Appendix 6. Section 5 is a short conclusions section.

2 Biochemical networks

We provide context for our results by relating them to those in Reed et al. (2017) and Ma et al. (2009). Since model systems form a proper subset of admissible systems, we emphasize the following: If infinitesimal homeostasis can only occur in a restricted form of an admissible system, then infinitesimal homeostasis can only occur in model systems with that same restricted form.

Common homeostatic mechanisms in cell metabolism include feedforward excitation Fig. 4a, substrate inhibition Fig. 5a, and product inhibition Fig. 6a. Another prevalent network topology that performs biochemical homeostasis is the negative feedback loop Fig. 7a. The first three motifs are taken from Reed et al. (2017). In these motifs nodes **X**, **Y**, **Z** are the names of chemical substrates and each straight arrow represents a flux coming into or going away from a substrate. Each substrate in these motifs degrades. Whereas, in the negative feedback motif from Ma et al. (2009), nodes denote enzymes that can have active and inactive forms. Unlike arrows in the first three motifs, here a positive arrow (e.g., $\mathbf{Z} \to \mathbf{Y}$) indicates the active state of enzyme **Z** can convert enzyme **Y** from its inactive to its active state. A negative arrow

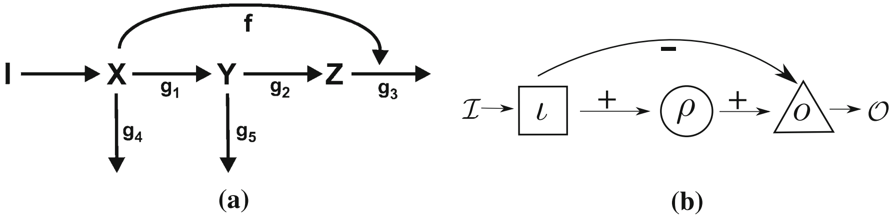

Fig. 4 Feedforward excitation: **a** Motif from Reed et al. (2017); **b** Input–output network with two simple paths from ι to o corresponding to the motif in (**a**). Arrows with plus signs are excitatory, whereas arrows with minus signs are inhibitory

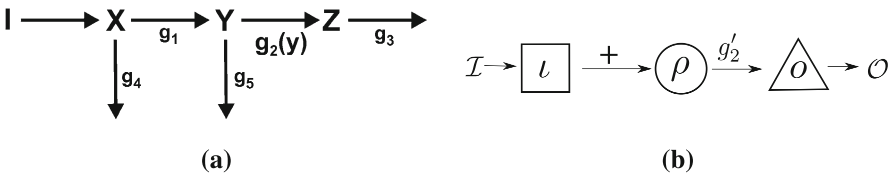

Fig. 5 Substrate inhibition: **a** Motif from Reed et al. (2017); **b** Input–output network with one simple path from ι to o corresponding to the motif in (**a**). The coupling $\iota \to \rho$ is excitatory, whereas the strength of the coupling $\rho \to o$ is given by g_2'

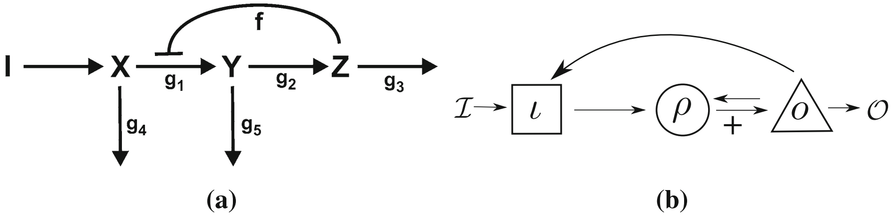

Fig. 6 Product inhibition: **a** Motif from Reed et al. (2017); **b** Input–output network with one simple path from ι to o corresponding to the motif in (**a**). Based on assumptions made in Reed et al. (2017), the coupling $\rho \to o$ is excitatory and the coupling $\iota \to \rho$ cannot be neutral

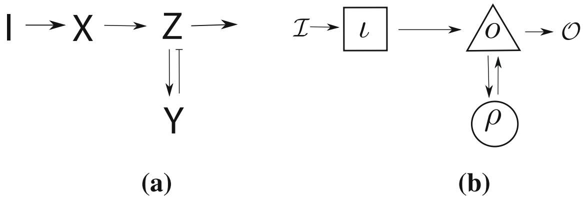

Fig. 7 Negative feedback loop: **a** Motif adapted from Ma et al. (2009); Unlike the arrows in Figs. 4, 5 and 6 that represent mass transition between substrates, these arrows represent activation and inactivation of one enzyme by another. **b** Input–output network with one simple path from ι to o corresponding to the motif in (**a**)

(e.g., $\mathbf{Y} \dashv \mathbf{Z}$) denotes the conversion of **Z** from its active to its inactive state by the enzyme **Y**.

The righthand pictures in these figures are the corresponding mathematical input–output diagrams. The biochemical notation relates to the mathematical notation as follows: the **X** substrate or enzyme is the ι node, the **Y** substrate or enzyme is the ρ node, and the **Z** substrate or enzyme is the o node. The biochemical concentrations (x, y, z) correspond to network variables (x_ι, x_ρ, x_o).

2.1 Feedforward excitation

The input–output network corresponding to the feedforward excitation motif in Fig. 4a is the network in Fig. 4b, which has two simple paths from the input node to the output node. In our discussion of two simple path networks, we observe that infinitesimal homeostasis is possible in such networks if (1.9) is satisfied; that is, the two simple paths from **X** to **Z** have opposite signs. In this motif, one simple path consists of two excitatory couplings from **X** to **Y** and from **Y** to **Z**, and hence has a positive sign. The other simple path is an excitatory coupling from **X** to the synthesis or degradation of **Z** and hence is an inhibitory simple path from **X** to **Z** having a negative sign. By Theorem 12, infinitesimal homeostasis is possible in feedforward excitation admissible systems (2.1, right), and can be computed by solving (1.8).

$$\begin{aligned} \dot{x} &= \mathcal{I} - g_1(x) - g_4(x) & \qquad \dot{x}_\iota &= f_\iota(x_\iota, \mathcal{I}) \\ \dot{y} &= g_1(x) - g_2(y) - g_5(y) & \dot{x}_\rho &= f_\rho(x_\iota, x_\rho) \\ \dot{z} &= g_2(y) - f(x)g_3(z) & \dot{x}_o &= f_o(x_\iota, x_\rho, x_o) \end{aligned} \tag{2.1}$$

Reed et al. (2017) showed that the model systems (2.1, left) for the feedforward excitation motif do indeed lead to infinitesimal homeostasis. Using (1.8) we reproduce their result by showing that infinitesimal homeostasis exists in the model systems when

$$f'(x_0) = \frac{g_1'(x_0)g_2'(y_0)}{(g_2'(y_0) + g_5'(y_0))g_3(z_0)} \tag{2.2}$$

where $X_0 = (x_0, y_0, z_0)$. Specifically, first compute

$$f_{o,x_\rho} = g_2'(y) \quad f_{\rho,x_\iota} = g_1'(x) \quad f_{o,x_\iota} = -f'(x)g_3(z) \quad f_{\rho,x_\rho} = -g_2'(y) - g_5'(y)$$

Equation (2.2) then follows directly from (1.8).

2.2 Substrate inhibition

Since the input–output network in Fig. 5b corresponding to the substrate inhibition motif in Fig. 5a contains only one simple path from ι to o, our results show that infinitesimal homeostasis is possible in an admissible system only through neutral coupling. Also, note that the coupling from $\mathbf{X}$ to $\mathbf{Y}$ is assumed to be excitatory ($g_1 > 0$) in the model system (2.3, left) for the substrate inhibition motif; that is, $f_{\rho,x_\iota} > 0$ in the admissible network system (2.3, right). It follows that neutral coupling is possible only in the $\rho \to o$ coupling; that is, $f_{o,x_\rho} = 0$.

$$\begin{aligned} \dot{x} &= \mathcal{I} - g_1(x) - g_4(x) & \qquad \dot{x}_\iota &= f_\iota(x_\iota, \mathcal{I}) \\ \dot{y} &= g_1(x) - g_5(y) - g_2(y) & \dot{x}_\rho &= f_\rho(x_\iota, x_\rho) \\ \dot{z} &= g_2(y) - g_3(z) & \dot{x}_o &= f_o(x_\rho, x_o) \end{aligned} \tag{2.3}$$

Hence, by Theorem 10 infinitesimal homeostasis can occur in this motif if and only if the coupling is neutral (that is, if $f_{o,x_\rho} = g_2' = 0$ at the equilibrium point). This observation agrees with the observation in Reed et al. (2017) that $\mathbf{Z}$ can exhibit infinitesimal homeostasis in the substrate inhibition motif if the infinitesimal homeostasis is built into the kinetics term g_2 between $\mathbf{Y}$ and $\mathbf{Z}$.

Reed et al. (2017) note that neutral coupling can arise from substrate inhibition of enzymes, enzymes that are inhibited by their own substrates. See the discussion in Reed et al. (2010). This inhibition leads to reaction velocity curves that rise to a maximum (the coupling is excitatory) and then descend (the coupling is inhibitory) as the substrate concentration increases. Infinitesimal homeostasis with neutral couplings arising from substrate inhibition often has important biological functions and has been estimated to occur in about 20% of enzymes Reed et al. (2010).

2.3 Product inhibition

Since the input–output network in Fig. 6b corresponding to the product inhibition motif in Fig. 6a contains only one simple path from ι to o, our results show that infinitesimal homeostasis is possible in an admissible system (2.4, right) only through neutral coupling. Also, the coupling from **Y** to **Z** is assumed to be excitatory in the model system (2.4, left) based on the biochemical network in Fig. 6a and used in Reed et al. (2017); that is, $f_{o,x_\rho} > 0$ in the admissible network system (2.4, right). It follows that neutral coupling is possible only in the $\iota \to \rho$ coupling; that is, $f_{\rho,x_\iota} = 0$.

$$\begin{aligned} \dot{x} &= \mathcal{I} - g_4(x) - f(z)g_1(x) \\ \dot{y} &= f(z)g_1(x) - g_2(y) - g_5(y) \\ \dot{z} &= g_2(y) - g_3(z) \end{aligned} \qquad\qquad \begin{aligned} \dot{x}_\iota &= f_\iota(x_\iota, x_o, \mathcal{I}) \\ \dot{x}_\rho &= f_\rho(x_\iota, x_\rho, x_o) \\ \dot{x}_o &= f_o(x_\rho, x_o) \end{aligned} \tag{2.4}$$

So, infinitesimal homeostasis is possible only if $f_{\rho,x_\iota} = g_1' f = 0$. However, in the Reed et al. (2017) model, $g_1 f$ is assumed to satisfy $g_1' f > 0$, which precludes infinitesimal homeostasis. It is shown in Reed et al. (2017) that homeostasis is possible in such systems if one chooses an $g_1(x) f(z)$ for which $g_1' f$ is close to zero. Moreover, such a choice is consistent with the biochemistry of product inhibition.

2.4 Negative feedback loop

Note that the input–output network in Fig. 7b corresponding to the negative feedback loop motif in Fig. 7a has only one simple path $\iota \to o$. It follows from our results that infinitesimal homeostasis is possible in the negative feedback loop if and only if the coupling $\iota \to o$ is neutral (Haldane) or the linearized internal dynamics of the regulatory node ρ is zero (null-degradation).

$$\begin{aligned} \dot{x} &= \mathcal{I} k_{\mathcal{I}x} \tfrac{1-x}{(1-x)+K_{\mathcal{I}x}} - F_x k'_{F_x} \tfrac{x}{x+K'_{F_x}} \\ \dot{y} &= z k_{zy} - F_y k'_{F_y} \\ \dot{z} &= x k_{xz} \tfrac{1-z}{(1-z)+K_{xz}} - y k'_{yz} \tfrac{z}{z+K'_{yz}} \end{aligned} \qquad\qquad \begin{aligned} \dot{x}_\iota &= f_\iota(x_\iota, \mathcal{I}) \\ \dot{x}_\rho &= f_\rho(x_\rho, x_o) \\ \dot{x}_o &= f_o(x_\iota, x_\rho, x_o) \end{aligned} \tag{2.5}$$

where $k_{\mathcal{I}x}$, $K_{\mathcal{I}x}$, F_x, k'_{F_x}, K'_{F_x}, k_{zy}, F_y, k'_{F_y}, k_{xz}, K_{xz}, k'_{yz}, K'_{yz} are 12 constants.

Each enzyme **X**, **Y**, **Z** in the feedback loop motif (Fig. 7) can have active and inactive forms. In the kinetic equations in the model the $\dot{y}$ equation does not depend on y and homeostasis can only be perfect homeostasis. However, this model is a simplification based on saturation in y Ma et al. (2009). In the original system $\dot{y}$ does depend on y and we expect standard null-degradation homeostasis to be possible in that system.

We note that stability of the equilibrium in this motif implies negative feedback. The Jacobian of (2.5, right) is

$$J = \begin{bmatrix} f_{\iota,x_\iota} & 0 & 0 \\ 0 & f_{\rho,x_\rho} & f_{\rho,x_o} \\ f_{o,x_\iota} & f_{o,x_\rho} & f_{o,x_o} \end{bmatrix}.$$

At null-degradation homeostasis ($f_{\rho,x_\rho} = 0$) it follows from linear stability that

$$f_{\iota,x_\iota} < 0, \qquad f_{o,x_o} < 0, \qquad f_{\rho,x_o} f_{o,x_\rho} < 0. \tag{2.6}$$

Conditions (2.6) imply that both the input node and the output node need to degrade and the couplings $\rho \to o$ and $o \to \rho$ must have opposite signs. This observation agrees with Ma et al. (2009) that homeostasis is possible in the network motif Fig. 7a if there is a negative loop between **Y** and **Z** and when the linearized internal dynamics of **Y** is zero.

Another biochemical example of null-degradation homeostasis can be found in (Ferrell 2016, Fig 2).

3 Infinitesimal homeostasis

We begin this section by showing that the output node must be downstream from the input node in any input–output network that admits infinitesimal homeostasis. We then use Cramer's rule to determine explicitly the derivative x_o' of the input–output function in three-node input–output networks.

Definition 7 Node q is *downstream* from node p if there exists a path from p to q.

Lemma 8 *In an n-node network, the existence of generic infinitesimal homeostasis implies that the output node o is downstream from the input node ι.*

Proof Let $\mathcal{U}(o)$ be the set of nodes upstream from o and assume that $\iota \notin \mathcal{U}(o)$. Note that the phase space associated with $\mathcal{U}(o)$ is a flow invariant subspace of phase space. Let $\dot{U} = G(U)$ be the restriction of the admissible vector field $\dot{X} = F(X, \mathcal{I})$ to phase space of $\mathcal{U}(o)$. The fact that input node ι is not upstream from o implies that G is independent of the input term $\mathcal{I}$. Let $U(\mathcal{I})$ equal the coordinates of $X(\mathcal{I})$ on $\mathcal{U}(o)$. Then $U(\mathcal{I})$ is a stable equilibrium of the vector field G and $U(\mathcal{I})$ is independent of $\mathcal{I}$. In addition, the input–output function is independent of $\mathcal{I}$. So, only in a very degenerate way can infinitesimal homeostasis occur. For instance, simple homeostasis and chairs are not possible. □

Next we compute x_o'. Begin by observing that the Jacobian of (1.3) is

$$J = \begin{bmatrix} f_{\iota,x_\iota} & f_{\iota,x_\rho} & f_{\iota,x_o} \\ f_{\rho,x_\iota} & f_{\rho,x_\rho} & f_{\rho,x_o} \\ f_{o,x_\iota} & f_{o,x_\rho} & f_{o,x_o} \end{bmatrix} \tag{3.1}$$

Note that J is invertible at $(X_0, \mathcal{I}_0)$ since the equilibrium X_0 at I_0 is assumed stable. Hence $\det(J) \neq 0$.

Lemma 9 *The input–output function $x_o(\mathcal{I})$ satisfies*

$$x_o' = \frac{1}{\det(J)} \det \begin{bmatrix} f_{\iota,x_\iota} & f_{\iota,x_\rho} & -f_{\iota,\mathcal{I}} \\ f_{\rho,x_\iota} & f_{\rho,x_\rho} & 0 \\ f_{o,x_\iota} & f_{o,x_\rho} & 0 \end{bmatrix} \tag{3.2}$$

Assume

$$f_{\iota,\mathcal{I}} \neq 0. \tag{3.3}$$

Then the input–output function x_o has a point of infinitesimal homeostasis if and only if

$$\mathcal{H} \equiv \det \begin{bmatrix} f_{\rho,x_\iota} & f_{\rho,x_\rho} \\ f_{o,x_\iota} & f_{o,x_\rho} \end{bmatrix} = f_{o,x_\rho} f_{\rho,x_\iota} - f_{o,x_\iota} f_{\rho,x_\rho} = 0. \tag{3.4}$$

Proof Implicit differentiation of (1.3) with respect to $\mathcal{I}$ yields the matrix system

$$J \begin{bmatrix} x_i' \\ x_\rho' \\ x_o' \end{bmatrix} = - \begin{bmatrix} f_{\iota,\mathcal{I}} \\ 0 \\ 0 \end{bmatrix} \tag{3.5}$$

Apply Cramer's rule to (3.5) to solve for the third coordinate x_o' yielding (3.2). By assumption, $f_{\iota,\mathcal{I}} \neq 0$. Hence, $\mathcal{I}_0$ is a point of infinitesimal homeostasis (that is, $x_o'(\mathcal{I}_0) = 0$) if and only if $\mathcal{H}(\mathcal{I}_0) = 0$, That is, (3.4) is valid. □

3.1 Infinitesimal homeostasis with neutral coupling and zero internal dynamics

Theorem 10 *Given an input–output three-node network. Suppose there is only one simple path from the input node to the output node. Then infinitesimal homeostasis implies neutral coupling or zero internal dynamics. Specifically,*

(a) $\iota \to o$*: infinitesimal homeostasis occurs if and only if* $f_{o,x_\iota} = 0$ *or* $f_{\rho,x_\rho} = 0$.
(b) $\iota \to \rho \to o$*: infinitesimal homeostasis occurs if and only if* $f_{o,x_\rho} = 0$ *or* $f_{\rho,x_\iota} = 0$.

Proof The proof proceeds in two parts.

(a) $\iota \to o$ and $\rho \not\to o$ or $\iota \not\to \rho$. If there is one simple path from ι to o and that path is the direct one $\iota \to o$, then the other simple path $\iota \to \rho \to o$ must not be present. Hence, at least one of the connections $\rho \to o$ or $\iota \to \rho$ must not be present. So, either $f_{o,x_\rho} \equiv 0$ or $f_{\rho,x_\iota} \equiv 0$. It follows from (3.4) that infinitesimal homeostasis occurs if and only if $f_{o,x_\iota} f_{\rho,x_\rho} = 0$, as desired.

(b) $\iota \to \rho \to o$ and $\iota \not\to o$. If there is one simple path from ι to o and that path is the indirect one $\iota \to \rho \to o$, then the direct path $\iota \to o$ must not be present. Hence $f_{o,x_\iota} \equiv 0$. It follows from (3.4) that infinitesimal homeostasis occurs if and only if $f_{\rho,x_\iota} f_{o,x_\rho} = 0$.

To summarize: infinitesimal homeostasis plus (a) implies neutral coupling in $\iota \to o$ or zero linearlized internal dynamics in node ρ and infinitesimal homeostasis plus (b) implies neutral coupling in either $\iota \to \rho$ or $\rho \to o$. □

An interpretation of Theorem 10 is that a three-node network with one simple path from input to output can have infinitesimal homeostasis only if that homeostasis is Haldane or *null-degradation*. The biological significance of this observation is discussed briefly in the subsection on substrate inhibition in Sect. 2 and expanded on in Reed et al. (2017).

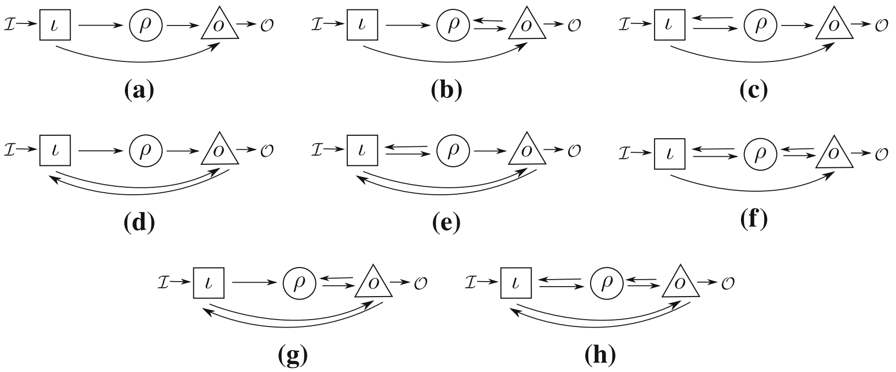

Fig. 8 Input–output networks with two simple paths. Each network contains a feedforward loop (Fig. 10b) as a subnetwork

3.2 Infinitesimal homeostasis with non-neutral coupling

Proposition 11 *Given a three-node input–output system with simple paths* $\iota \to o$ *and* $\iota \to \rho \to o$. *Homeostasis* $x_o'(\mathcal{I}_0) = 0$ *is equivalent to* (3.4) *at* $\mathcal{I}_0$. *Moreover, generically the couplings* $\iota \to o$, $\iota \to \rho$, $\rho \to o$ *are either excitatory or inhibitory and the internal dynamics of node* ρ *is nonzero.*

Proof The validity of (3.4) follows directly from Lemma 9. Moreover since the couplings $\iota \to o$, $\iota \to \rho$, and $\rho \to o$ are in the network, these couplings as well as the internal dynamics f_{ρ,x_ρ} can be perturbed arbitrarily in admissible systems and are generically nonzero. □

Theorem 12 *Up to node relabeling there are eight input–output three-node networks that can support infinitesimal homeostasis with couplings that are either excitatory or inhibitory and these networks have a feedforward loop as a subnetwork. See Fig. 8. Moreover, infinitesimal homeostasis implies that the couplings are restricted by*

$$\mathrm{sgn}(f_{\rho,x_\iota} f_{o,x_\rho})\mathrm{sgn}(f_{o,x_\iota}) = \mathrm{sgn}(f_{\rho,x_\rho}). \tag{3.6}$$

Proof As shown in Theorem 10 infinitesimal homeostasis in any network with a single simple path from the input node ι to the output node o leads to neutral coupling or zero internal dynamics. So we can assume that the network has two simple paths from the input node to the output node. In particular all arrows $\iota \to \rho$, $\iota \to o$, $\rho \to o$ are in the network. Hence the feedforward loop is a subnetwork. Generically, these couplings are excitatory or inhibitory and the internal dynamics $f_{\rho,x_\rho} \neq 0$.

It follows from Proposition 11 that

$$f_{o,x_\rho} f_{\rho,x_\iota} = f_{o,x_\iota} f_{\rho,x_\rho}.$$

Since the four entries are generically nonzero, the identity (3.6) is valid. □

4 Chair homeostasis

Codimension one homeostasis occurs when on variation of a parameter, such as a rate constant, a double degeneracy is found. In the context of infinitesimal homeostasis the only such example is the chair point. Nijhout and Reed (2014) and Nijhout et al. (2014) argue that in fact the most important way that homeostasis appears in applications (in particular in biochemical networks) is through a *chair*. The proofs of results in this section require complicated calculations and are given in the Appendix 7.

The singularity theory notion of an *infinitesimal chair* has normal form $\mathcal{I}^3$. Using elementary catastrophe theory, Golubitsky and Stewart (2017) observe that infinitesimal chairs satisfy the defining conditions

$$x_o'(\mathcal{I}_0) = x_o''(\mathcal{I}_0) = 0 \tag{4.1}$$

and the nondegeneracy condition

$$x_o'''(\mathcal{I}_0) \neq 0 \tag{4.2}$$

where $'$ denotes differentiation with respect to $\mathcal{I}$.

Recall from (3.4) in Lemma 9 that infinitesimal homeostasis is given by solving $\mathcal{H}(\mathcal{I}) = 0$ where

$$\mathcal{H} \equiv f_{o,x_\rho} f_{\rho,x_\iota} - f_{o,x_\iota} f_{\rho,x_\rho}.$$

In Proposition 13 we present the defining and nondegeneracy conditions for an infinitesimal chair in terms of derivatives of $\mathcal{H}$.

Proposition 13 *The input–output function $x_o(\mathcal{I})$ associated to (1.3) satisfies the defining and nondegeneracy conditions of an infinitesimal chair at $\mathcal{I}_0$ if and only if*

$$\mathcal{H} = \mathcal{H}' = 0 \tag{4.3}$$

and

$$\mathcal{H}'' \neq 0. \tag{4.4}$$

Remark 14 No simplification of the calculations needed to find an infinitesimal chair, in the structural homeostasis case, beyond that given in Proposition 13 seems possible. Explicit calculation of infinitesimal chairs for the model equations of feedforward excitation reproduced in (2.1, left) are given in Reed et al. (2017). These calculations can be reproduced using Proposition 13.

In Theorems 15 and 16 we explicitly compute the defining and nondegeneracy conditions for an infinitesimal chair in terms of derivatives of f_ι, f_ρ, f_o when the network has only one simple path from ι to o. We recall that chair points for structural homeostasis were found in Reed et al. (2017) by explicitly evaluating $\mathcal{H}$ and $\mathcal{H}'$. The corresponding general result for structural homeostasis is quite complicated and will not be pursued here.

Theorem 15 *Given a three-node input–output network with only one simple path from input node to output node and that path is $\iota \to o$.*

Suppose this path has a neutral coupling at an infinitesimal homeostasis point. Then the defining conditions for a chair (4.1) are equivalent to

$$f_{o,x_\iota} = f_{o,x_\iota x_\iota} = 0$$

and the chair nondegeneracy condition (4.2) is valid if and only if

$$f_{o,x_\iota x_\iota x_\iota} \neq 0.$$

Theorem 16 *Given a three-node input–output network with only one simple path from input node to output node and that path is $\iota \to \rho \to o$.*

Suppose this path has neutral coupling in $\iota \to \rho$. Then the defining conditions for a chair (4.1) are

$$f_{\rho,x_\iota} = f_{\rho,x_\iota x_\iota} = 0$$

and the nondegeneracy condition for a chair (4.2) is

$$f_{\rho,x_\iota x_\iota x_\iota} \neq 0.$$

Suppose this path has neutral coupling in $\rho \to o$. Then the defining conditions for a chair (4.1) are

$$f_{o,x_\rho} = f_{o,x_\rho x_\rho} = 0$$

and the nondegeneracy condition for a chair (4.2) is

$$f_{o,x_\rho x_\rho x_\rho} \neq 0.$$

Remark 17 It follows from Theorems 15 and 16 that having a chair in Haldane homeostasis implies that the kinetic term itself has a chair at that infinitesimal homeostasis point.

5 Conclusions and future developments

In this paper we showed how infinitesimal homeostasis can arise in three-node networks. We assume there is an input node ι and an output node o. If the network contains two simple paths from ι to o, then infinitesimal homeostasis is given by feedforward excitation. If the network contains only one simple path from ι to o, then infinitesimal

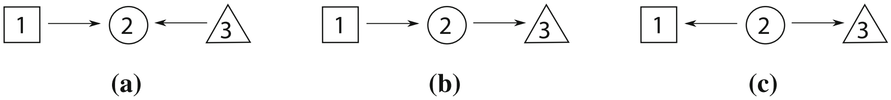

Fig. 9 Inhomogeneous three-cell networks with two arrows

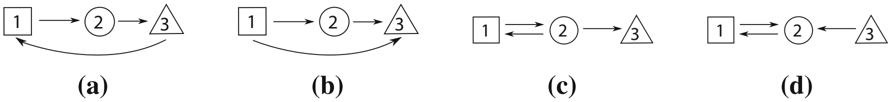

Fig. 10 Inhomogeneous three-cell networks with three arrows

homeostasis is given by neutral coupling (as shown in substrate and product inhibition) or zero internal dynamics (as shown in negative feedback loop).

There are two natural questions:

(a) Can this classification of one input parameter infinitesimal homeostasis be extended to networks with $n \geq 4$ nodes?

(b) Golubitsky and Stewart (2018) discuss how singularity theory can be used to classify multi-input/one output infinitesimal homeostasis. Is there a network theory for multiple inputs?

There are 199 four-node networks and 9364 five-node networks.[3] These numbers indicate some of the complexity of question (a). Preliminary work indicates that the answer to (a) is yes. Attempts to answer question (b) should begin with analyses of three and four-node networks.

6 Appendix: Classification of three-node networks

Theorem 18 *Up to node relabeling there are 13 three-node networks that are pictured in Figs. 9, 10, 11 and 12.*

It follows from Theorem 18 that there are $78 = 6 \times 13$ possible choices of three-node input–output networks.

Proof There are either 1, 2, or 3 transitive components in a three-node network.

If there are three transitive components, then there are four possible networks and they are given in networks a–c in Fig. 9 and network b in Fig. 10.

If there are two transitive components, then one of the components has two nodes each connected to the other. The third node connects to one node in the two-node component in either direction (networks c and d in Fig. 10) or to both nodes in the two-node component in either direction (networks c and d in Fig. 11).

The remaining networks have one transitive component. There are two possibilities: the network has a unidirectional cycle $1 \to 2 \to 3$ or two cycles $1 \leftrightarrow 2$ and $2 \leftrightarrow 3$. There is one possibility in the last case and that is network a in Fig. 11. The possible networks with a three-cycle are network a in Fig. 10, network b in Fig. 11 and networks

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[3] See the Online Encyclopedia of Integer Sequences at http://oeis.org/A003085.

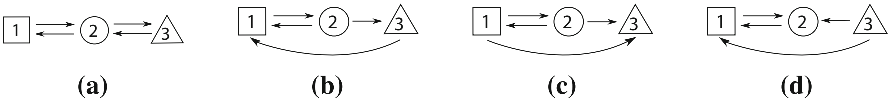
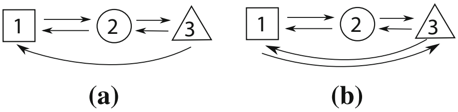


Fig. 11 Inhomogeneous three-cell networks with four arrows

Fig. 12 Inhomogeneous three-cell networks with five and six arrows



a and b in Fig. 12. These four are distinguished by how many additional arrows each has. □

Remark 19 Tyson and Novak (2010) classify three-node networks with two types of coupling: excitatory and inhibitory. In this paper we will also allow neutral coupling, which complicates the classification, but we only consider networks that support infinitesimal homeostasis, which simplifies the classification.

7 Appendix: Proofs of results in Sect. 4

Lemma 20 *Suppose* $x_o'(\mathcal{I}) = \varphi(\mathcal{I}) y_o'(\mathcal{I})$ *where* $\varphi(\mathcal{I}_0) \neq 0$. *Then for all* $m \geq 1$

$$x_o'(\mathcal{I}_0) = \cdots = x_o^{[m]}(\mathcal{I}_0) = 0 \quad \textit{if and only if} \quad y_o'(\mathcal{I}_0) = \cdots = y_o^{[m]}(\mathcal{I}_0) = 0.$$

Proof The proof is straightforward by induction. □

Proof of Proposition 13 Recall from (3.2) that

$$x_o' = \frac{1}{\det(J)} \det \begin{bmatrix} f_{\iota,x_\iota} & f_{\iota,x_\rho} & -f_{\iota,\mathcal{I}} \\ f_{\rho,x_\iota} & f_{\rho,x_\rho} & 0 \\ f_{o,x_\iota} & f_{o,x_\rho} & 0 \end{bmatrix} = -\frac{f_{\iota,\mathcal{I}}}{\det(J)} \mathcal{H} \tag{7.1}$$

where the Jacobian J is given by (3.1) and $\det(J) \neq 0$. It follows from Lemma 20 that the first few derivatives of x_o' vanish at $\mathcal{I}_0$ if and only if the same number of derivatives of $\mathcal{H}$ vanish at $\mathcal{I}_0$. Therefore, $\mathcal{I}_0$ satisfies the defining conditions of an infinitesimal chair if and only if $x_o' = x_o'' = 0$ if and only if (4.3) is satisfied; that is, $\mathcal{H} = \mathcal{H}' = 0$. Therefore, $\mathcal{I}_0$ satisfies the nondegeneracy condition of an infinitesimal chair if and only if $x_o''' \neq 0$ if and only if (4.4) is satisfied; that is, $\mathcal{H}'' \neq 0$. □

Proofs of Theorems 15 and 16 will use the following lemma. Recall that a is a *backward arrow if either the head of a is ι or the tail of a is o.*

Lemma 21 *Given a three-node input–output network $\mathcal{G}$ where each node has nonzero internal dynamics. Computation of the infinitesimal homeostasis point in $\mathcal{G}$ is not affected by deletion of backward arrows from $\mathcal{G}$.*

Proof Let $\tilde{\mathcal{G}}$ be the reduced network obtained by removing all backward arrows from the original network $\mathcal{G}$. It follows that the Jacobian matrix $\tilde{J}$ for the reduced network is lower triangular with eigenvalues $f_{\ell,x_\ell} \neq 0$ at the infinitesimal homeostasis point $\mathcal{I}_0$, where $\ell = \iota, \rho, o$. Hence $\det(\tilde{J}) \neq 0$ at $\mathcal{I}_0$.

Next we show the two networks have the same condition for computating infinitesimal homeostasis. It follows from Lemma 9 that the linearized couplings f_{ι,x_ρ}, f_{ι,x_o}, and f_{ρ,x_o} associated to backward arrows do not appear in $\mathcal{H}$ given by (3.4). The formula (7.1) is therefore valid for both networks with the only difference being between $\det(J)$ and $\det(\tilde{J})$, both of which are nonzero at the infinitesimal homeostasis point $\mathcal{I}_0$. It follows that

$$x_o'(\mathcal{I}) = \varphi(\mathcal{I})\tilde{x}_o'(\mathcal{I})$$

where x_o, $\tilde{x}_o$ are the input–output functions of the original network and the associated reduced network with no backward arrows, and $\varphi(\mathcal{I}) = \det(\tilde{J})/\det(J)$ with $\varphi(\mathcal{I}_0) \neq 0$. Thus, Lemma 20 implies that removing backward arrows has no effect on computing the infinitesimal homeostasis point. □

Proof of Theorem 15 By Proposition 13, an infinitesimal chair point occurs at $\mathcal{I}_0$ if and only if $\mathcal{H}(\mathcal{I}_0) = \mathcal{H}'(\mathcal{I}_0) = 0$ and $\mathcal{H}''(\mathcal{I}_0) \neq 0$. The proof therefore proceeds by showing that

$$\mathcal{H} = \mathcal{H}' = 0;\ \mathcal{H}'' \neq 0 \ \text{ if and only if } \ f_{o,x_\iota} = f_{o,x_\iota x_\iota} = 0;\ f_{o,x_\iota x_\iota x_\iota} \neq 0. \tag{7.2}$$

Since by assumption there is a neutral coupling, generically linearlized internal dynamics of nodes in $\mathcal{G}$ are nonzero. By Lemma 21, we can assume there is no backward arrow in the admissible system:

$$\begin{aligned} f_\iota(x_\iota, \mathcal{I}) &= 0 \\ f_\rho(x_\iota, x_\rho) &= 0 \\ f_o(x_\iota, x_\rho, x_o) &= 0 \end{aligned} \tag{7.3}$$

Thus

$$\begin{bmatrix} f_{\iota,x_\iota} & 0 & 0 \\ f_{\rho,x_\iota} & f_{\rho,x_\rho} & 0 \\ f_{o,x_\iota} & f_{o,x_\rho} & f_{o,x_o} \end{bmatrix} \begin{bmatrix} x_\iota' \\ x_\rho' \\ x_o' \end{bmatrix} = \begin{bmatrix} -f_{\iota,\mathcal{I}} \\ 0 \\ 0 \end{bmatrix}$$

It follows that $x_\iota' = -f_{\iota,\mathcal{I}}/f_{\iota,x_\iota} \neq 0$ and

$$x_\rho' = -f_{\rho,x_\iota}x_\iota'/f_{\rho,x_\rho} \tag{7.4}$$

Since there is only one simple path from ι to o given by $\iota \to o$, the other simple path $\iota \to \rho \to o$ must not be present. It follows that

$$f_{o,x_\rho}f_{\rho,x_\iota} \equiv 0. \tag{7.5}$$

Therefore the definition of $\mathcal{H}$ in (3.4) reduces to

$$\mathcal{H} \equiv -f_{o,x_\iota} f_{\rho,x_\rho}.$$

Since generically $f_{\rho,x_\rho} \neq 0$, by Lemma 20

$$\mathcal{H} = \mathcal{H}' = 0;\ \ \mathcal{H}'' \neq 0 \quad \text{if and only if} \quad f_{o,x_\iota} = f'_{o,x_\iota} = 0;\ \ f''_{o,x_\iota} \neq 0.$$

We therefore verify (7.2) by showing

$$f'_{o,x_\iota} = 0 \quad \text{if and only if} \quad f_{o,x_\iota x_\iota} = 0 \tag{7.6}$$

and

$$f''_{o,x_\iota} \neq 0 \quad \text{if and only if} \quad f_{o,x_\iota x_\iota x_\iota} \neq 0. \tag{7.7}$$

Direct computation shows that

$$\begin{aligned} f'_{o,x_\iota} &\equiv f_{o,x_\iota x_\iota} x'_\iota + f_{o,x_\iota x_\rho} x'_\rho + f_{o,x_\iota x_o} x'_o \\ &\equiv f_{o,x_\iota x_\iota} x'_\iota + f_{o,x_\iota x_o} x'_o \\ &= f_{o,x_\iota x_\iota} x'_\iota \end{aligned} \tag{7.8}$$

where the second identity in (7.8) holds because

$$f_{o,x_\iota x_\rho} x'_\rho = -f_{o,x_\iota x_\rho} f_{\rho,x_\iota} (x'_\iota / f_{\rho,x_\rho}) \equiv 0$$

by (7.4) and (7.5). Since $x'_\iota \neq 0$, (7.6) holds. Differentiating f'_{o,x_i} in (7.8) with respect to $\mathcal{I}$ leads to

$$\begin{aligned} f''_{o,x_\iota} &\equiv (f_{o,x_\iota x_\iota} x'_\iota + f_{o,x_\iota x_o} x'_o)' \\ &= f_{o,x_\iota x_\iota x_\iota} x'^2_\iota \end{aligned} \tag{7.9}$$

because $x'_o(\mathcal{I}_0) = x''_o(\mathcal{I}_0) = 0$ and $f_{o,x_\iota x_\iota x_\rho} x'_\rho \equiv 0 \equiv f_{o,x_\iota x_o x_\rho} x'_\rho$ by (7.4) and (7.5). It follows that (7.7) is true. □

Proof of Theorem 16 This theorem has two cases; we only prove the first as the arguments for the second case are identical.

By Proposition 13, an infinitesimal chair point occurs at $\mathcal{I}_0$ if and only if $\mathcal{H}(\mathcal{I}_0) = \mathcal{H}'(\mathcal{I}_0) = 0$ and $\mathcal{H}''(\mathcal{I}_0) \neq 0$. The proof therefore proceeds by showing that

$$\mathcal{H} = \mathcal{H}' = 0;\ \ \mathcal{H}'' \neq 0 \text{ if and only if } f_{o,x_\rho} = f_{o,x_\rho x_\rho} = 0;\ \ f_{o,x_\rho x_\rho x_\rho} \neq 0. \tag{7.10}$$

Since the couplings $\iota \to \rho \to o$ exist the coupling $\iota \not\to o$ does not; that is, $f_{o,x_\iota} \equiv 0$. We also note that generically linearized internal dynamics f_{ι,x_ι}, f_{ρ,x_ρ}, f_{o,x_o} are nonzero. By Lemma 21, we can assume there is no backward arrow in the admissible system:

$$\begin{aligned} f_\iota(x_\iota, \mathcal{I}) &= 0 \\ f_\rho(x_\iota, x_\rho) &= 0 \\ f_o(x_\rho, x_o) &= 0 \end{aligned} \tag{7.11}$$

Thus

$$\begin{bmatrix} f_{\iota,x_\iota} & 0 & 0 \\ f_{\rho,x_\iota} & f_{\rho,x_\rho} & 0 \\ 0 & f_{o,x_\rho} & f_{o,x_o} \end{bmatrix} \begin{bmatrix} x'_\iota \\ x'_\rho \\ x'_o \end{bmatrix} = \begin{bmatrix} -f_{\iota,\mathcal{I}} \\ 0 \\ 0 \end{bmatrix}$$

It follows that $x'_\iota = -f_{\iota,\mathcal{I}}/f_{\iota,x_\iota} \neq 0$ and

$$x'_\rho = -f_{\rho,x_\iota} x'_\iota / f_{\rho,x_\rho}. \tag{7.12}$$

Since f_{o,x_ι} is identically zero

$$\mathcal{H} \equiv f_{o,x_\rho} f_{\rho,x_\iota}. \tag{7.13}$$

Hence, $\mathcal{H} = 0$ if and only if $f_{o,x_\rho} = 0$ or $f_{\rho,x_\iota} = 0$.

In the first case $f_{o,x_\rho} = 0$ and $f_{\rho,x_\iota} \neq 0$. By Lemma 20

$$\mathcal{H} = \mathcal{H}' = 0;\ \mathcal{H}'' \neq 0 \quad \text{if and only if} \quad f_{o,x_\rho} = f'_{o,x_\rho} = 0;\ f''_{o,x_\rho} \neq 0.$$

We therefore verify (7.10) by showing

$$f'_{o,x_\rho} = 0 \quad \text{if and only if} \quad f_{o,x_\rho x_\rho} = 0 \tag{7.14}$$

and

$$f''_{o,x_\rho} \neq 0 \quad \text{if and only if} \quad f_{o,x_\rho x_\rho x_\rho} \neq 0. \tag{7.15}$$

Direct computation shows

$$\begin{aligned} f'_{o,x_\rho} &\equiv f_{o,x_\rho x_\iota} x'_\iota + f_{o,x_\rho x_\rho} x'_\rho + f_{o,x_\rho x_o} x'_o \\ &\equiv f_{o,x_\rho x_\rho} x'_\rho + f_{o,x_\rho x_o} x'_o \\ &= f_{o,x_\rho x_\rho} x'_\rho \end{aligned} \tag{7.16}$$

where the second identity holds because $f_{o,x_\iota} \equiv 0$. By (7.12) $x'_\rho \neq 0$ and hence (7.14) holds.

Differentiating f'_{o,x_ρ} in (7.16) leads to

$$\begin{aligned} f''_{o,x_\rho} &\equiv (f_{o,x_\rho x_\rho} x'_\rho)' + (f_{o,x_\rho x_o} x'_o)' \\ &= (f_{o,x_\rho x_\rho} x'_\rho)' \\ &= f_{o,x_\rho x_\rho x_\rho} x'^2_\rho \end{aligned}$$

because $x'_o = x''_o = 0$ at $\mathcal{I}_0$ and $f_{o,x_\iota} \equiv 0$. It follows that (7.15) is true. Hence this case is verified. $\square$

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