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Flow-inducing networks

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***Abstract*—We consider *flow-inducing networks*, a class of models that are well-suited to describe important biochemical systems, including the MAPK pathway and the interactions at the trans-Golgi network. A flow-inducing network is given by the interconnection of subsystems (modules), each associated with a stochastic state matrix whose entries depend on the state variables of other modules. This results in an overall nonlinear system; when the interactions are modelled as mass action kinetics, the overall system is bilinear. We provide preliminary results concerning the existence of single or multiple equilibria and their positivity. We also show that instability phenomena are possible and that entropy is not a suitable Lyapunov function. The simplest non-trivial module is the *duet*, a second order system whose variables represent the concentrations of a species in its activated and inhibited state: under mass action kinetics assumptions, we prove that (i) a negative loop of duets has a unique equilibrium that is unconditionally stable and (ii) a positive loop of duets has either a unique stable equilibrium on the boundary or two equilibria, of which one is unstable on the boundary and one is strictly positive and stable; both properties (i) and (ii) hold regardless of the number of duets in the loop.**

***Index Terms*—Biomolecular systems, Network analysis and control, Systems biology**

I. Introduction and Motivating Examples

THE mathematical study of biological models [1], [17], [30] helps us gain insight into natural phenomena and identify recurring motifs [1] that explain the complex and astoundingly resilient behaviour of biological systems, in spite of severe uncertainties and variability. Biological and biochemical systems are extremely robust [6] due to their particular structure, regardless of specific parameter values: hence, it is possible to identify *structural* (parameter-free) properties, such as stability [7], [8], oscillations and multistationarity [9], [10], signed steady-state behaviours [23]. In particular, the theory of chemical reaction networks [18], [21], [26] (see also the tutorial [3]), has provided celebrated structural results on the stability, existence and uniqueness of equilibria, such as the zero-deficiency and the one-deficiency theorems [19], [20], which have fostered a lot of work on stability [2], [11], [25], [31] and multiple (stable) equilibria [4], [5], [13], [14], [16].

Here we consider *flow-inducing networks* arising from the interaction among compartmental [28] modules (i.e., subsystems that group positive variables subject to mass conservation), where the flows among variables in each module are tuned by the value of variables in other modules. The variables in each module evolve according to a stochastic state matrix (a Markov chain), whose entries depend on flow-inducing signals coming from other modules, without retroactivity effects [15].

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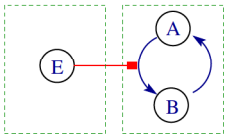

Figure 1: Graph representing the simplified enzymatic reaction in Eq. (1): the simplest flow-inducing system with a biochemical interpretation.

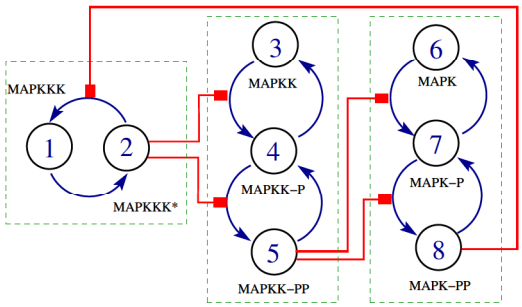

Figure 2: The MAPK pathway with negative feedback.

A flow-inducing network can be associated with a *graph*, where state variables (species concentrations) are represented by nodes, flows by arcs (blue pointed arrows) connecting nodes, and flow-inducing signals by meta-arcs (red hammer-head arrows) connecting a node to an arc, while nodes associated with variables in the same module are grouped by dotted green boxes (see, for instance, Figs. 1 and 2).

The *flow-inducing* framework is suited to describe the behaviour of some (bio)chemical systems. In fact, it captures the essential aspect of *catalysis*: given two (bio)chemical species A and B that are reacting with each other, the presence of species E (the *catalyst*, or *enzyme* in biocatalysis) in the reaction environment can lower the activation energy required for the reaction and boost the reaction rate, thus inducing a higher flow between A and B, *without being consumed* by the reaction. To a first approximation, this phenomenon can be represented by the simplified biochemical reaction network

$$E + A \xrightarrow{k_{ae}} E + B, \quad B \xrightarrow{k_b} A, \tag{1}$$

corresponding to the graph in Fig. 1, where module 1 includes the enzyme E and module 2 includes species A and B. The presence of E induces the flow from A to B in module 2, while the flow from B to A is spontaneous.

Remark 1: The reaction $E + A \xrightarrow{k} E + B$ is obtained by neglecting the dynamics of the enzyme-substrate complex C in the actual reactions $A+E \rightleftharpoons C \xrightarrow{k} B+E$. This approximation may lead to inaccurate predictions [29], but is widely accepted for the qualitative analysis of large-scale systems. ◇

Also more complex models that are typically adopted to represent fundamental biochemical systems, such as the MAPK pathway and the interactions at the trans-Golgi network, fit

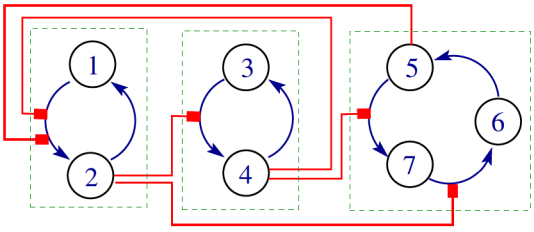

Figure 3: Graph representation of the trans-Golgi model A.

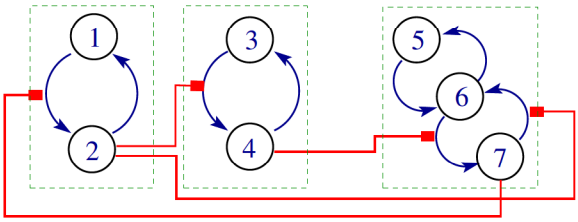

Figure 4: Graph representation of the trans-Golgi model B.

into the flow-inducing framework. This is illustrated by the following examples, which present models taken directly from the biochemical literature.

Example 1: **The MAPK pathway** plays an essential role in regulating cell growth, differentiation and apoptosis in several living organisms. When mitogens (growth factors) bind to the cell surface, Mitogen-Activated Protein Kinases propagate their signal to the cell nucleus through a pathway of three proteins activated in series: binding mitogens activate MAPKKK, which in turn activates MAPKK, which finally activates MAPK, in charge of triggering gene expression in the cell nucleus. Both MAPKKK and MAPKK are activated through double phosphorylation [22], [27]. The cascade equations with a negative loop from MAPK to MAPKKK are

$$\begin{cases} \dot{x}_1 = \alpha x_2 x_8 - \beta x_1 \\ \dot{x}_2 = -\alpha x_2 x_8 + \beta x_1 \end{cases} \begin{cases} \dot{x}_3 = -\gamma x_2 x_3 + \delta x_4 \\ \dot{x}_4 = \gamma x_2 x_3 - \delta x_4 - \varepsilon x_2 x_4 + \zeta x_5 \\ \dot{x}_5 = \varepsilon x_2 x_4 - \zeta x_5 \end{cases} \begin{cases} \dot{x}_6 = -\eta x_5 x_6 + \vartheta x_7 \\ \dot{x}_7 = \eta x_5 x_6 - \vartheta x_7 - \mu x_5 x_7 + \nu x_8 \\ \dot{x}_8 = \mu x_5 x_7 - \nu x_8 \end{cases} \tag{2}$$

where x_1 = [MAPKKK] inactive, x_2 = [MAPKKK*] active, x_3 = [MAPKK], x_4 = [MAPKK-P], x_5 = [MAPKK-PP], x_6 = [MAPK], x_7 = [MAPK-P], x_8 = [MAPK-PP]; the suffix -P denotes phosphorylation, -PP double phosphorylation. Since $\dot{x}_1 + \dot{x}_2 = 0$, $\dot{x}_3 + \dot{x}_4 + \dot{x}_5 = 0$ and $\dot{x}_6 + \dot{x}_7 + \dot{x}_8 = 0$, system (2) can be seen as the interconnection of three mass preserving modules, as shown in the graph in Fig. 2. No flow occurs between any two variables belonging to different modules. However, some flows within the modules are tuned by variables belonging to other modules: x_2 (in the first module) induces the flows from x_3 to x_4 and from x_4 to x_5 in the second module; x_5 (in the second module) induces the flows from x_6 to x_7 and from x_7 to x_8 in the third module; a negative-feedback is due to x_8 (in the third module), which induces the flow from x_2 to x_1 in the first module. ◇

Example 2: In mammalian cells, **PKD-CERT interactions at the trans-Golgi network** (TGN) regulate the process that transfers secretory proteins to the cell membrane by means of various types of transport vesicles. PKD, a protein kinase that regulates the fission of transport vesicles, interacts with the kinase PI4KIIIβ and with CERT, a transfer protein that transports ceramide from the Endoplasmatic Reticulum (where it is synthesised) to the TGN. Weber et al. [32] recently proposed two different models to describe this complex interaction of proteins and lipids, still only partially understood. If we neglect the inputs (included in [32] to experimentally fit the models) and assume that exogenous production and self-degradation compensate each other for each of the variables, then model A ("short distance shuttle") becomes

$$\begin{cases} \dot{x}_1 = -x_1 h(x_4, x_5) - \alpha_1 x_1 + \alpha_2 x_2 \\ \dot{x}_2 = x_1 h(x_4, x_5) + \alpha_1 x_1 - \alpha_2 x_2 \end{cases} \begin{cases} \dot{x}_3 = \kappa x_4 - x_3 g_1(x_2) \\ \dot{x}_4 = -\kappa x_4 + x_3 g_1(x_2) \end{cases} \begin{cases} \dot{x}_5 = \lambda x_6 - x_5 g_4(x_4) \\ \dot{x}_6 = -\lambda x_6 + x_7 g_2(x_2) \\ \dot{x}_7 = x_5 g_4(x_4) - x_7 g_2(x_2) \end{cases} \tag{3}$$

while model B ("neck swinging") becomes

$$\begin{cases} \dot{x}_1 = -x_1 g_7(x_7) - \alpha_1 x_1 + \alpha_2 x_2 \\ \dot{x}_2 = x_1 g_7(x_7) + \alpha_1 x_1 - \alpha_2 x_2 \end{cases} \begin{cases} \dot{x}_3 = \kappa x_4 - x_3 g_1(x_2) \\ \dot{x}_4 = -\kappa x_4 + x_3 g_1(x_2) \end{cases} \begin{cases} \dot{x}_5 = -\nu x_5 + \lambda x_6 \\ \dot{x}_6 = \nu x_5 - \lambda x_6 - x_6 g_4(x_4) + x_7 g_2(x_2) \\ \dot{x}_7 = x_6 g_4(x_4) - x_7 g_2(x_2) \end{cases} \tag{4}$$

where x_1 = [PKD], x_2 = [PKDpDAG], x_3 = [PI4KIIIβ], x_4 = [PI4KIIIβp]; x_5 = [CERTaER] in model A and x_5 = [CERTa] in model B; x_6 = [CERTpER] in model A and x_6 = [CERTp] in model B; x_7 = [CERTaTGN] in model A and x_7 = [CERTaERTGN] in model B. In [32], the functions are $g_i(z) = a_i z/(z+b_i)$ and $h(z,y) = a_j y a_i \frac{z}{z+b_i}/(b_j + y a_i \frac{z}{z+b_i})$, with a_i, b_i, a_j, $b_j > 0$. In both models, there are three compartmental modules: $\dot{x}_1 + \dot{x}_2 = 0$, $\dot{x}_3 + \dot{x}_4 = 0$ and $\dot{x}_5 + \dot{x}_6 + \dot{x}_7 = 0$. Flows involve only variables belonging to the same module. Some flows are tuned by variables in other modules: for instance, in model A (corresponding to the graph in Fig. 3) x_2, in the first module, induces the flow from x_7 to x_6 in the third module, which exhibits an overall circular flow; in model B (corresponding to the graph in Fig. 4) x_2, in the first module, induces the flow from x_7 to x_6 in the third module, which has a two-step flow structure, with x_6 acting as an intermediate stage between x_5 and x_7. ◇

Can we exploit the structure of flow-inducing networks to assess stability, uniqueness and positivity of equilibria?

In this paper, we introduce the class of flow-inducing networks (Section II). We give some preliminary results on the existence of single or multiple equilibria and on their positivity, and show that entropy, often successfully chosen as a candidate Lyapunov function for mass-action-kinetics networks [3], [19], [25], is not a suitable choice here (Section III).

Under mass action kinetics assumptions, we give promising results concerning loops of *duets*, modules with two variables, which can represent the concentrations of a species in its activated and inhibited state. We show that a negative loop of duets has a unique equilibrium that is unconditionally stable, regardless of the choice of the parameter values (Section IV-A) and that a positive loop of duets can either have a unique stable equilibrium, which lies on the boundary (i.e., the state vector has at least one zero component), or two equilibria, one unstable on the boundary, and one strictly positive and stable; it is possible to ensure the existence of a stable positive equilibrium (thus activating the system) by increasing the total species concentrations in the modules (Section IV-B). Remarkably, both results hold for an arbitrary number of duets involved in the loop. Section IV-C shows that these results may no longer hold when different kinetics are considered. Concluding comments are provided in Section V.

II. Model Description and Graph Representation

Consider the aggregate of N subsystems (modules), each associated with a componentwise nonnegative state vector $z_k(t) \in \mathbb{R}^{n_k}$, $z_k(t) \geq 0$ for all t. The overall system, which we call *flow-inducing network*, has the state vector

$$x(t) = [z_1(t)^\top \; z_2(t)^\top \dots z_N(t)^\top]^\top \in \mathbb{R}^n,$$

with $n = \sum_{k=1}^N n_k$. Let $\tilde{z}_k = [z_1^\top \dots z_{k-1}^\top \; z_{k+1}^\top \dots z_N^\top]^\top$ denote the complement vector to z_k, obtained from x by removing z_k. The kth module has dynamics

$$\dot{z}_k(t) = A_k(\tilde{z}_k) z_k(t), \tag{5}$$

where $A_k(\tilde{z}_k)$ is a Metzler matrix (its off-diagonal entries are nonnegative) whose columns have zero sum: $\bar{1}^\top A_k(\tilde{z}_k) = \bar{0}$, where $\bar{1}$ denotes the all-ones vector and $\bar{0}$ the all-zeros vector of the appropriate size. Hence, system (5) is compartmental.

In the following, all constants are assumed to be positive.

Assumption 1: The off-diagonal entries of $A_k(\tilde{z}_k)$ are either nonnegative constants or nonnegative smooth functions, strictly increasing in each argument, which are zero if and only if at least one of the arguments is zero. ◇

Remark 2: When the entries of $A_k(\tilde{z}_k)$ are linear increasing functions, we obtain biochemical reaction networks where kinetics follow the law of mass action. The main stability results in Section IV are valid for this case. ◇

Since $A_k(\tilde{z}_k)$ are Metzler matrices and $d/dt(\bar{1}^\top z_k(t)) = \bar{1}^\top A(\tilde{z}_k) z_k = 0$, the following property holds.

Proposition 1: The solution $z_k(t)$ of subsystem (5) with nonnegative initial conditions $z_k(0) \geq 0$ remains nonnegative and satisfies mass conservation constraints:

$$z_k(t) \in \Xi_k(p_k) = \{\xi \in \mathbb{R}^{n_k} : \bar{1}^\top \xi = p_k, \; \xi \geq 0\}, \; \forall \, t, \tag{6}$$

where $p_k = \bar{1}^\top z_k(0)$. □

The overall system solution $x(t)$ lies in the Cartesian product

$$\Xi = \Xi_1(p_1) \times \Xi_2(p_2) \times \dots \times \Xi_N(p_N),$$

depending on the initial conditions. For chemical reaction networks, Ξ is known as the *stoichiometric compatibility class*.

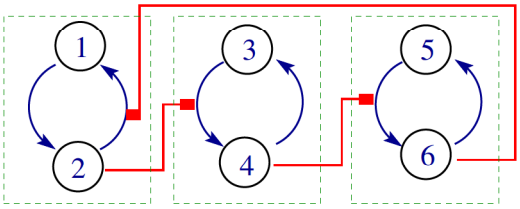

Figure 5: Graph representation of the flow-inducing network (7), with three duets and a negative feedback loop.

If $A_k(\tilde{z}_k)$ is irreducible for any *fixed* $\tilde{z}_k$, then subsystem (5) is stable within $\Xi_k(p_k)$: $z_k(t)$ converges to the equilibrium corresponding to the Frobenius eigenvector of $A_k(\tilde{z}_k)$, associated with the zero eigenvalue. Yet stability of the overall system, given by the interaction of the modules, is not guaranteed.

A flow-inducing network can be represented as a *graph* $\mathcal{G}(\mathcal{N}, \mathcal{A}, \mathcal{M})$, where

- the *nodes* in $\mathcal{N} = \{1, \dots, n\}$ are associated with the *state variables* $x_1, \dots, x_n$ and partitioned into *clusters* $\mathcal{N}_k$ of size n_k, representing the *modules* z_k, $k = 1, \dots, N$;
- the *arcs* in $\mathcal{A} \subseteq \{(i,j) : i,j \in \mathcal{N}_k \text{ for some } k\}$ represent *flows* and connect pairs of nodes in the same cluster, where i is the departing node and j the arrival node;
- the *meta-arcs* in $\mathcal{M} \subseteq \{(i,(k,h)) : i \in \mathcal{N}, (k,h) \in \mathcal{A}\}$ represent *flow-inducing signals* and connect nodes to arcs.

Flows associated with arcs (i,j) are linear functions of the variable associated with the departing node: $f(i,j) = k_{ij} x_i$; if the linearity coefficient depends on a variable x_k in another module, with $k_{ij} = k_{ij}(x_k)$ satisfying Assumption 1, then a meta-arc connects the kth node to the flow arc (i,j).

For example, the graph in Fig. 5 corresponds to the flow-inducing network with equations

$$\begin{cases} \dot{x}_2 = \alpha_1 x_1 - g_2(x_6) x_2 = -\dot{x}_1 \\ \dot{x}_4 = g_3(x_2) x_3 - \beta_2 x_4 = -\dot{x}_3 \\ \dot{x}_6 = g_5(x_4) x_5 - \beta_3 x_6 = -\dot{x}_5 \end{cases} \tag{7}$$

The module vectors are: $z_1 = [x_1 \; x_2]^\top$, $z_2 = [x_3 \; x_4]^\top$ and $z_3 = [x_5 \; x_6]^\top$. System (7) is an aggregate of three *duets*: second order modules whose variables represent the concentrations of a species in its activated and inhibited state. In the first module, for instance, the flow $\alpha_1 x_1$ from node 1 to node 2 depends linearly on x_1; the flow $g_2(x_6) x_2$ from node 2 to node 1 depends linearly on x_2, but possibly nonlinearly on x_6; there is no flow between x_6 and x_2. Assuming linearity with respect to all variables leads to mass action kinetics: $g_2(x_6) x_2 = \beta_1 x_6 x_2$, $g_3(x_2) x_3 = \alpha_2 x_2 x_3$, $g_5(x_4) x_5 = \alpha_3 x_4 x_5$.

III. Equilibria and Stability Analysis

Since $z_k(t) \in \Xi_k(p_k)$, the system evolution is constrained in a compact set, hence there is always an equilibrium point [24]. How many equilibria are there? Do they lie on the boundary (i.e., do they have at least one zero entry)? To avoid trivial conclusions, we assume all modules are strongly connected.

Assumption 2: For any pair of nodes $i,j \in \mathcal{N}_k$, $k \in \{1, \dots, N\}$, there is an oriented flow path from i to j. ◇

Definition 1: An *influencing variable* of x_i is a variable x_j such that either $(j,i) \in \mathcal{A}$ or $(j,(k,i)) \in \mathcal{M}$ for some k, i.e., a variable involved in positive terms in the equation of $\dot{x}_i$. $\diamond$

Influencing variables can be associated either with flows or with flow-inducing signals. For instance, in Fig. 5, x_2 (flow) and x_6 (flow-inducing signal) are influencing x_1.

Proposition 2: Consider an aggregate of subsystems (5), under Assumption 2. If a variable is 0 at the equilibrium, then at least one of its influencing variables must be 0 as well. $\square$

For instance, system (7) cannot have boundary equilibria, as shown in Section IV-A. Conversely, the network in Fig. 6 can have a boundary equilibrium with $x_2 = x_4 = x_6 = 0$, which can be unique or not, depending on the stoichiometric compatibility class where the system is confined (see Section IV-B).

Remark 3: Based on Proposition 2, a procedure to find boundary equilibria can be easily implemented by using graph methods (see for instance [5], [13], [14]).

The stability analysis of an equilibrium for the considered class of systems is non-trivial. One solid reason for this claim is that entropy, often adopted as a Lyapunov function for chemical reaction networks, is not suitable in general for flow-inducing networks, even under mass action kinetics assumptions. Entropy is defined as

$$H(x) = \sum_{i=1}^{n} \left[x_i \log\left(\frac{x_i}{\bar{x}_i}\right) - x_i \right],$$

where $\bar{x}_i$ are the equilibrium values, and has a local minimum at $x_i = \bar{x}_i$. Its gradient is $\nabla H(x) = \left[\log\left(\frac{x_1}{\bar{x}_1}\right) \ \log\left(\frac{x_2}{\bar{x}_2}\right) \ \dots \ \log\left(\frac{x_n}{\bar{x}_n}\right)\right]$.

Proposition 3: The flow-inducing system

$$\begin{cases} \dot{x}_2 = -\mu x_2 + \mu x_1 x_4 = -\dot{x}_1 \\ \dot{x}_4 = -\nu x_4 + \nu x_2 x_3 = -\dot{x}_3 \end{cases}$$

has a stable equilibrium $\bar{x}_i = 1$, for all i. Still, for $\nu = 10$, $\mu = 0.1$ and $x_1 + x_2 = x_3 + x_4 = 2$, the Lyapunov derivative of the system entropy H can be positive. $\square$

Proof: Stability will be proved in Theorem 1 for a more general case. The entropy function has Lyapunov derivative

$$\begin{aligned} \dot{H}(x) &= \mu \log\left(\frac{2-x_2}{x_2}\right)[x_2 - x_4(2-x_2)] \\ &+ \nu \log\left(\frac{2-x_4}{x_4}\right)[x_4 - x_2(2-x_4)]. \end{aligned}$$

For $x_2 = 0.1$ and $x_4 = 0.3$, we get $\dot{H} \approx 2.12 > 0$. $\blacksquare$

Hence, we need other candidate Lyapunov functions.

IV. Loops of Duets: Mass Action Analysis

This section studies negative and positive loops of an arbitrary number of duets (modules with two variables, representing the concentrations of a species in its activated and inhibited state), under mass action kinetics assumptions.

Aggregates of ducts are fully described by the equations of $\dot{x}_k$ with k even, $k = 2, 4, \dots, n$. In fact, since $x_{k-1} + x_k = p_{\frac{k}{2}}$ is a constant, $\dot{x}_{k-1} = -\dot{x}_k$.

We show that, for negative loops, a positive equilibrium exists and is stable; for positive loops, either a single stable equilibrium exists on the boundary, or a pair of equilibria exist, one unstable on the boundary and one positive and stable.

A. The negative loop of duets has a unique positive unconditionally stable equilibrium

Under mass action kinetics assumptions, the negative loop of N duets (see Fig. 5 for the graph representation in the case $N = 3$) corresponds to the system of equations

$$\begin{cases} \dot{x}_2 = \alpha_1(p_1 - x_2) - \beta_1 x_2 x_n, \\ \dot{x}_k = \alpha_{\frac{k}{2}}(p_{\frac{k}{2}} - x_k)x_{k-2} - \beta_{\frac{k}{2}} x_k, \ k = 4, 6, \dots, n, \end{cases} \tag{8}$$

and $\dot{x}_{k-1} = -\dot{x}_k$, with $k = 2, 4, \dots, n$. Variable x_k activates x_{k+2}, for all even $2 \le k \le n-2$, while x_n inhibits x_2.

To show that system (8) has a unique equilibrium that is positive and stable, we rely on the following lemma.

Lemma 1: A polynomial of the form

$$p(s) = (s+\sigma_1)(s+\sigma_2)\dots(s+\sigma_m) \pm \sigma'_1\sigma'_2\dots\sigma'_m,$$

with $0 < \sigma'_k < \sigma_k$ for $k = 1, 2, \dots, m$, is Hurwitz (namely, it does not admit roots with nonnegative real part). $\square$

Proof: By contradiction, let $\xi + j\omega$ be a root with $\xi \ge 0$:

$$(j\omega+\xi+\sigma_1)(j\omega+\xi+\sigma_2)\dots(j\omega+\xi+\sigma_{\frac{n}{2}}) = \mp\sigma'_1\sigma'_2\dots\sigma'_{\frac{n}{2}}.$$

If we take the square modulus of both sides, we get

$$[\omega^2+(\xi+\sigma_1)^2][\omega^2+(\xi+\sigma_2)^2]\dots[\omega^2+(\xi+\sigma_{\frac{n}{2}})^2] = {\sigma'_1}^2{\sigma'_2}^2\dots{\sigma'_{\frac{n}{2}}}^2,$$

which is impossible, since $\sigma'_k < \sigma_k$. $\blacksquare$

Theorem 1: System (8) admits a unique equilibrium that is positive and unconditionally Hurwitz stable. $\square$

Proof: The equilibrium conditions are

$$\begin{aligned} \bar{x}_2 &= \frac{\alpha_1 p_1}{\alpha_1 + \beta_1 \bar{x}_n} \doteq \phi_2(\bar{x}_n), \\ \bar{x}_k &= \frac{\alpha_{\frac{k}{2}} p_{\frac{k}{2}} \bar{x}_{k-2}}{\alpha_{\frac{k}{2}} \bar{x}_{k-2} + \beta_{\frac{k}{2}}} \doteq \phi_k(\bar{x}_{k-2}), \ k = 4, 6, \dots, n. \end{aligned}$$

Function ϕ_2 is decreasing and converges to 0 as $\bar{x}_n \to \infty$. If we compose the functions, $\bar{x}_n = \phi_n \circ \phi_{n-2} \circ \dots \circ \phi_4(\bar{x}_2) \doteq \phi(\bar{x}_2)$, we see that ϕ is an increasing function that converges to a constant value when $\bar{x}_2 \to \infty$. Hence there is a single intersection, corresponding to positive values of $\bar{x}_2$, $\bar{x}_n$.

Consider the equilibrium conditions

$$\alpha_{\frac{k}{2}}(p_{\frac{k}{2}} - x_k) = \beta_{\frac{k}{2}} \frac{x_k}{x_{k-2}}, \ k = 4, 6, \dots, n,$$

and denote $D_k = \alpha_{\frac{k}{2}} x_{k-2} + \beta_{\frac{k}{2}}$. Then, the Jacobian is

$$\begin{bmatrix} -(\alpha_1+\beta_1 x_n) & 0 & 0 & \dots & -\beta_1 x_2 \\ \beta_2 \frac{x_4}{x_2} & -D_4 & 0 & \dots & 0 \\ 0 & \beta_3 \frac{x_6}{x_4} & -D_6 & \dots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & \beta_{\frac{n}{2}} \frac{x_n}{x_{n-2}} & -D_n \end{bmatrix}$$

and the corresponding characteristic polynomial

$$\begin{aligned} p(s) &= (s+\alpha_1+\beta_1 x_n)(s+\alpha_2 x_2+\beta_2)\dots(s+\alpha_{\frac{n}{2}} x_{n-2}+\beta_{\frac{n}{2}}) \\ &+ \beta_1 x_n \beta_2 \beta_3 \dots \beta_{\frac{n}{2}} \end{aligned}$$

is Hurwitz in view of Lemma 1. $\blacksquare$

B. Positive equilibria (if any) of positive loops of duets are unconditionally stable

Under mass action kinetics assumptions, the positive loop of N duets (see Fig. 6 for the graph representation in the case $N = 3$) corresponds to the system of equations

$$\dot{x}_k = \alpha_{\frac{k}{2}}(p_{\frac{k}{2}} - x_k)x_{k-2} - \beta_{\frac{k}{2}}x_k, \ k = 2, 4, \dots, n, \qquad (9)$$

where $x_0 \doteq x_n$. The system has a boundary equilibrium with

$$\bar{x}_2 = \bar{x}_4 = \dots = \bar{x}_n = 0. \qquad (10)$$

Does it admit other equilibria?

Theorem 2: In addition to the equilibrium (10), system (9) admits a unique positive equilibrium if and only if

$$\prod_{i=1}^{\frac{n}{2}} \frac{\alpha_i p_i}{\beta_i} > 1. \qquad (11)$$

If the positive equilibrium exists, it is Hurwitz stable. □

Proof: The equilibrium conditions can be written as

$$\bar{x}_k = \frac{p_{\frac{k}{2}}\alpha_{\frac{k}{2}}\bar{x}_{k-2}}{\beta_{\frac{k}{2}} + \alpha_{\frac{k}{2}}\bar{x}_{k-2}} \doteq \phi_k(\bar{x}_{k-2}), \ k = 2, 4, \dots, n.$$

All the functions ϕ_k are increasing and concave (they have decreasing derivative). By composing these functions, we get

$$\bar{x}_n = \phi_n \circ \phi_{n-2} \circ \dots \circ \phi_2(\bar{x}_n) \doteq \phi(\bar{x}_n)$$

The derivative of ϕ is the product $\phi' = \phi'_n\phi'_{n-2}\dots\phi'_2$, which is positive, decreasing and such that $\lim_{x_n\to\infty}\phi'(x_n) = 0$. Then $\phi(x_n)$ is increasing and concave. Moreover, $\phi(0) = 0$ (corresponding to the boundary equilibrium). Computing the derivative of ϕ as the product of the derivatives of the functions ϕ_k, we get $\phi'(0) = \prod_{i=1}^{\frac{n}{2}} \frac{\alpha_i p_i}{\beta_i}$. There are two possibilities.

a) If $\phi'(0) > 1$, then the concave function $\psi(x_n) = \phi(x_n) - x_n$ is positive in a right neighborhood of the origin and becomes negative after a certain value (in fact, $\lim_{x_n\to\infty}\psi'(x_n) = -1$), hence it admits a single positive root.

b) If $\phi'(0) \leq 1$, then $\psi(x_n)$ is always negative for positive x_n, hence the system cannot admit a positive equilibrium.

For a strictly positive equilibrium, the conditions are

$$\alpha_{\frac{k}{2}}(p_{\frac{k}{2}} - x_k) = \beta_{\frac{k}{2}}\frac{x_k}{x_{k-2}}.$$

Then, the Jacobian can be written as

$$\begin{bmatrix} -D_2 & 0 & 0 & \dots & \beta_1\frac{x_2}{x_n} \\ \beta_2\frac{x_4}{x_2} & -D_4 & 0 & \dots & 0 \\ 0 & \beta_3\frac{x_6}{x_4} & -D_6 & \dots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & \beta_{\frac{n}{2}}\frac{x_n}{x_{n-2}} & -D_n \end{bmatrix},$$

with $D_k = \alpha_{\frac{n}{2}}x_{n-2} + \beta_{\frac{n}{2}}$, and its characteristic polynomial

$$\begin{aligned} p(s) &= (s + \alpha_1 x_n + \beta_1)(s + \alpha_2 x_2 + \beta_2)\dots(s + \alpha_{\frac{n}{2}}x_{n-2} + \beta_{\frac{n}{2}}) \\ &- \beta_1\beta_2\dots\beta_{\frac{n}{2}} \end{aligned}$$

is Hurwitz in view of Lemma 1. ■

Theorem 3: When $\prod_{i=1}^{\frac{n}{2}} \frac{\alpha_i p_i}{\beta_i} < 1$, system (9) admits a unique equilibrium (10), on the boundary, which is stable. □

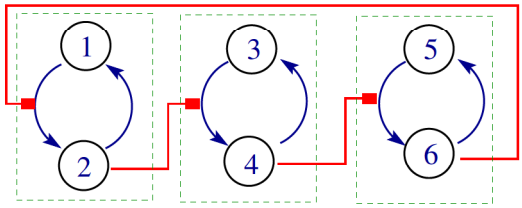

Figure 6: Graph representation of a flow-inducing network with three duets and a positive feedback loop.

Proof: Since inequality (11) is strictly violated, the system cannot admit the positive equilibrium in view of Theorem 2. The boundary equilibrium has the Jacobian

$$\begin{bmatrix} -\beta_1 & 0 & 0 & \dots & \alpha_1 p_1 \\ \alpha_2 p_2 & -\beta_2 & 0 & \dots & 0 \\ 0 & \alpha_3 p_3 & -\beta_3 & \dots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & \alpha_{\frac{n}{2}} p_{\frac{n}{2}} & -\beta_{\frac{n}{2}} \end{bmatrix},$$

corresponding to the characteristic polynomial

$$p(s) = (s + \beta_1)(s + \beta_2)\dots(s + \beta_{\frac{n}{2}}) - \alpha_1 p_1 \alpha_2 p_2 \dots \alpha_{\frac{n}{2}} p_{\frac{n}{2}}.$$

By assumption $\prod_{i=1}^{\frac{n}{2}} \alpha_i p_i < \prod_{i=1}^{\frac{n}{2}} \beta_i$. Hence, Lemma 1 ensures that the polynomial is Hurwitz.

Since the equilibrium is on the boundary, the linearisation argument is not enough in principle. However, the Jacobian is a Metzler matrix: its dominant Frobenius eigenvalue is real and negative, and all others have a smaller real part. The function

$$V(x_2, x_4, \dots, x_n) = v_1 x_2 + v_2 x_4 + \dots v_{\frac{n}{2}} x_n,$$

where $v_i > 0$ is the ith component of the left Frobenius eigenvector, is a co-positive Lyapunov function for the linearised system. Hence V is a local co-positive Lyapunov function for the nonlinear system as well. This guarantees Hurwitz stability of the equilibrium. ■

Remark 4: When inequality (11) holds, adopting the same arguments as in the proof of Theorem 3, it can be shown that the boundary equilibrium is unstable, because the constant term in $p(s)$ is negative and the Frobenius eigenvalue is positive. The condition (11) is biologically relevant: an additional external input designed to increase or decrease the quantities p_i can switch on or off (activate or inactivate) the system by introducing or removing the positive stable equilibrium. ◇

C. Non-mass-action kinetics

The results in the previous subsections are no longer valid when the entries of matrices $A_k(\tilde{z}_k)$ are not linear. Michaelis-Menten functions, $[A_k(\tilde{z}_k)]_{ij} = \frac{\alpha_{ij}\tilde{z}_{k,h}}{\beta_{ij}+\tilde{z}_{k,h}}$, and Hill functions with cooperativity index m, $[A_k(\tilde{z}_k)]_{ij} = \frac{\alpha_{ij}(\tilde{z}_{k,h})^m}{(\beta_{ij})^m+(\tilde{z}_{k,h})^m}$, are widespread in biochemical models and satisfy Assumption 1, hence can be included in our framework. However, as an example, the next result shows that our stability result for a negative loop of duets fails when $A_k(\tilde{z}_k)$ has nonlinear entries.

Example 3: For some choices of the functions g_k, the constants α_i and the initial conditions, the unique equilibrium of system (7) can be unstable. Indeed, if $p_i = 2$, $\alpha_i = 1$ for $i = 1, 2, 3$ and functions $g_k(x)$ are chosen so that $g_k(1) = 1$,

then the unique equilibrium is $\bar{x}_i = 1$ for all $i \in \{1, \dots, 6\}$. Since $x_1 = p_1 - x_2$, $x_3 = p_2 - x_4$ and $x_5 = p_3 - x_6$, we consider the Jacobian of the system with x_2, x_4, x_6 only, computed at the equilibrium:

$$J = \begin{bmatrix} -[\alpha_1 + g_2(\bar{x}_6)] & 0 & -g_2'(\bar{x}_6)\bar{x}_2 \\ g_3'(\bar{x}_2)(p_2 - \bar{x}_4) & -[\alpha_2 + g_3(\bar{x}_2)] & 0 \\ 0 & g_5'(\bar{x}_4)(p_3 - \bar{x}_6) & -[\alpha_3 + g_5(\bar{x}_4)] \end{bmatrix}$$

If the g_k's are Hill functions of the form

$$g_k(x) = \frac{2x^m}{x^m + 1}, \ m \in \mathbb{N},$$

then $g_k(1) = 1$, $g_k'(1) = m/2$ and the resulting characteristic polynomial $p(s) = (s+2)^3 + m^3/8$ has roots with positive real part for integer $m \geq 9$. $\diamond$

V. Conclusions and Future Work

We have proposed *flow-inducing networks*, a class of systems that is relevant in modelling (bio)chemical reaction networks where a species can boost a reaction among other species without being affected by the reaction.

Flow-inducing networks have peculiar properties, worth investigating. At least one equilibrium always exists, but it is not necessarily stable, even when it is unique. If there cannot be boundary equilibria (whose existence can be ruled out by exploring the system graph), then at least a positive equilibrium must exist. Entropy cannot be employed as a Lyapunov function, even under mass action kinetics. Under mass action kinetics assumptions, stronger results have been derived for loops of duets (second-order modules): regardless of the number of duets involved in the loop, (i) any negative loop of duets has a unique positive unconditionally stable equilibrium, while (ii) a positive loop of duets has either a single boundary equilibrium or two equilibria, one on the boundary and one positive and unconditionally stable.

There are several interesting open problems. For instance:

- Flow-inducing networks with mass action kinetics appear to be quite robust: we have not been able so far to find a case where a unique positive equilibrium is not stable. Is stability of a positive equilibrium always guaranteed with mass action kinetics? This problem is related to the stability of a general class of bilinear systems appearing also in a stochastic consensus problem [12].
- Are there other topologies (e.g., loops of third-order modules) for which general stability results can be provided?
- Can these systems admit multiple positive equilibria?
- Consider the generalisation $\dot{z}_k = A_k(\tilde{z}_k, z_k)z_k$, where the entries of A_k can be monotonic functions (either increasing or decreasing) of the local state variables as well. We conjecture that the proposed stability results about positive and negative loops can be extended to this case, under suitable assumptions.

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