

## Preface

This project has taken me longer than I would have liked, but exactly as long as I needed. I thank those that allowed me extra time to finish and I thank Hil Meijer for observing and accepting that I needed it. Moreover, I thank him for his continuous efforts to be a good supervisor, even when the project seemed lost. I also want to thank those that kept me going in difficult times; that is, Lilian Spijker for all the good advise, and my friends for offering support, different places to study and plenty of comic relief. I will always remember the stem cell called Bob.

# Two Nodes Fighting For a Win: Bistability and Stability Loss in Dynamics on GRN-like Networks 

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#### Abstract

One of the problems at the heart of osteoarthritis is chondrocyte hypertrophy. A study modelling the dynamics of chondrocyte hypertrophy showed three stable states: a SOX9-positive state linked to stable chondrocytes, a RUNX2-positive state linked to hypertrophy and a null-state linked to apoptosis. The question arose why the system is bi/multistable and why the RUNX2-state is reached. Through analysis of small networks similar to the large network used in the previously mentioned study, it was found that mutual inhibition seems to lie at the heart of bi/multistability.


Keywords: Osteoarthritis (OA), cell differentiation, bistability, saddle-node bifurcations, nonlinear dynamics, bifurcation analysis

## 1 Introduction

Osteoarthritis (OA) is a common, painful disease characterised by deterioration of cartilage in joints. One of the problems at the heart of osteoarthritis is chondrocyte hypertrophy; the chondrocytes differentiate into a type of cell that normally leads to bone formation. A lot of research has been done on how to repress chondrocyte hypertrophy, but the exact mechanism underlying chondrocyte hypertrophy is still unclear. [1]. Also, repressing hypertrophy does not replace the already damaged cartilage. Here tissue engineering could prove useful. The source in [1] and others propose using chondrocytes differentiated from human induced pluripotent stem cells (hiPSCs). The trouble is that this method has a low yield, and chondrocytes tend to become hypertrophic [2], which is exactly what we were trying to solve. Why do those chondrocytes become hypertrophic instead of remaining stable?

Stable chondrocytes are associated with the transcription factor SOX9 and hypertrophic chondrocytes with RUNX2 [1]. Expression of those transcription factors is regulated by many molecules and complexes, like other transcription factors, which interactions can be summarised in and modelled by a gene regulation network (GRN). Somehow that network drives the chondrocyte to differentiate to a state where it expresses RUNX2, a hypertrophic state.

In a study by Kerkhofs et al. ([3],[4]) the GRN shown in Figure 1 was used to model chondrocyte hypertrophy. More specifically, Kerkhofs modelled the dynamics of activation and interaction of part of the genes and proteins (and complexes) generally believed to govern differentiation of chondrocytes in the growth plate from a proliferating state to a hypertrophic state. Three states were found: RUNX2-positive, SOX9-positive and NONE, which was interpreted as apoptosis [3],[4].


Figure 1: The GRN used in [4]


Figure 2: Waddington's epigenetic landscape [5]

The RUNX2-positive state is assumed to be the hypertrophic state, that is, the state to be avoided in OA. Hence the question: why is it reached? For this, we need some understanding of cell differentiation. Waddington proposed an epigenetic landscape with valleys and ridges, a visual representation of stable equilibria and saddles, as in Figure 2. One might think of the chondrocytes as sitting in their nice SOX9 equilibrium on the landscape, where a RUNX2 equilibrium exists at the other side of a saddle. Then a perturbation might cause the landscape to change, so that the chondrocyte rolls towards the RUNX2 equilibrium, unable to get back. That describes the disappearance of the stable SOX9 state, much like the disappearance of a node by a saddle-node bifurcation in a dynamical system [6]. That is, parameter changes cause the SOX9-node and the saddle between the two stable states to coalesce and disappear. Why does that happen? Can we find something inside the network to explain that?

To find an answer to those questions, we dive into the dynamics on the network and pose the following research question. What causes bistability of a dynamical system based on a GRN, and what causes it to disappear?

The complete network used in Kerkhofs' study, as shown in Figure 1, is rather large and complex and therefore difficult to analyse when dynamics are considered on every node. That is why we shift our attention to smaller networks of a similar nature, leading to three sub-questions. We start with two-node networks. How do the dynamics on those networks behave? From there, what can be said about dynamics on three-node networks containing the two-node patterns? Moreover, what other patterns can be found?

## 2 Model description

Before we can analyse our networks, it is good to explain what those networks represent and what networks are of interest. Next, we need to specify the quantity used to model gene expression, and capture its dynamics in a model.

### 2.1 Networks

The networks in this study represent a GRN. A GRN models genes and proteins as nodes in a graph, with two types of interactions between nodes: activation/stimulation (denoted by a black arrow in Figure 1) and inhibition (denoted by a red arrow with square head). A stimulated gene is transcribed more, an inhibited gene is transcribed less. An activated protein is a functional one, an inhibited protein does not function. Factor A is said to
activate factor $B$ if $A$ somehow stimulates the formation of active $B$. Factor $C$ is said to inhibit factor B if C somehow negatively influences the activity of B .

The small models in this study are networks of two or three nodes, interacting through activation and inhibition. Those nodes guide a 'fight for dominance' of two imaginary factors. Figure 3 is an example of such a network.


Figure 3: Two nodes (circles) with a nonzero base level of activation (+-sign) inhibiting each other and activating competing factors (squares). Activation is depicted by black arrows and inhibition by red arrows with a square head.

For now the focus will be on dynamics on the nodes rather than the factors. Concretely, this means investigating the cases where node 1 activates A and node 2 activates B .

### 2.2 Dynamics

Now for the dynamical model. A common way of modelling gene expression in a cell is by considering activity of the factors in the GRN, a number between 0 and 1 , representing some qualitative measure of the amount of active protein or the rate at which a gene is transcribed [7]. Dynamics in this activity are governed by a GRN.

Activation of a factor leads to an active factor when activation is larger than a certain activation threshold $x_{0}$. The link between activation (some function $f$ of the activity of the activating nodes) and increase in activity of the activated node is often described by a sigmoid $S(x)$ where $S(x) \approx 0$ for $x \leq 0$ and $S(x) \approx 1$ for $x \geq 1$. In this study, the logistic function was used:

$$
\begin{equation*}
S(x)=\frac{1}{1+e^{-k\left(x-x_{0}\right)}}, \tag{1}
\end{equation*}
$$

where $x_{0}$ is taken 0.5 , precisely between 0 and 1 to avoid bias, and $k=10$, large enough so that $S(0) \approx 0, S(1) \approx 1$. This function is not exactly 0 for zero activation $(x=0)$, but close enough for our purposes.

Inhibition is taken as negative activation. It follows that inhibition only influences activation; if there is no activation, the input to the sigmoid is negative, but $S(x)=0$ for negative $x$. Hence, factor C is said to inhibit factor B if C somehow negatively influences the activation of B . Other cases of inhibition where C directly influences the formation of active $B$, such as $C$ directly inactivating $B$ or catalysing a reaction where active $B$ is a reactant, are considered as break down of B . Breakdown of B is modelled as having a rate proportional to activity of B , where factor C is neglected. In this study, that breakdown rate is taken equal to 1 .

So far we have seen that activity of a factor is influenced by activation, inhibition and breakdown. For modelling reasons we add another possibility, namely constant external activation, that is, constant activation from outside the GRN; the GRN does not give a complete picture, and this way some factors can always be activated without specifying
the source. Such constant activation is also referred to as a 'base level of activation' (like in the description of Figure 3).

The above can be summarised in a differential equation. The derivative of the activity $y$ is given by

$$
\begin{equation*}
y^{\prime}(t)=-y+S(f(x)) \tag{2}
\end{equation*}
$$

where $S$ is the logistic equation as in (1), $x$ is a vector of activity of the activating nodes and $f$ a function using the GRN structure to relate activity of the activating nodes to the total activation power. In GRNs, proteins can act in complexes, for example, which is often modelled by multiplying their activity. In this study, however, $f$ is taken a linear function of $x$.

The equation in (2) might then be written as

$$
\begin{equation*}
y^{\prime}(t)=-y+S\left(a_{0}+a_{1} x_{1}+\ldots+a_{n} x_{n}\right) \tag{3}
\end{equation*}
$$

where $a_{0}$ is a parameter denoting the strength of external activation, $x_{1}, \ldots, x_{n}$ are variables denoting the activity values of the $n$ nodes interacting with $y$ and $a_{1}, \ldots, a_{n}$ are parameters denoting the strength of interactions, which have a positive sign in case of activation and a negative sign in case of inhibition.

Not all interactions described in a GRN happen at the same time scale. For now, however, we will neglect that and focus on network dynamics on one time scale, unless it leads to interesting results.

As an example of a complete model, the network in Figure 3 can be described by the equations

$$
\left\{\begin{array}{l}
x_{1}^{\prime}(t)=-x_{1}+S\left(a_{0}-a_{1} x_{2}\right)  \tag{4}\\
x_{2}^{\prime}(t)=-x_{2}+S\left(b_{0}-b_{1} x_{1}\right)
\end{array}\right.
$$

where $a_{0}, a_{1}, b_{0}$ and $b_{1}$ are strictly positive parameters.

### 2.3 Some restrictions to the models of interest

Not all models following from the model description above are interesting to study. If models can be reduced to smaller models, it is the smaller models that are interesting. Therefore, a set of restrictions, say criteria for an interesting model, are in place.

1. Networks with nodes merely relaying an interaction, later referred to as 'messenger nodes', are avoided. Those nodes can be left out.
2. Every node in the network receives a form of activation. It is either activated by itself or other nodes, or is externally activated, which is modelled by inserting a base level of activation. Else the node can be left out.
3. Networks cannot be separated into smaller networks between which communication is one-directional. Networks that can, can be reduced and analysed part by part.

## 3 Two Nodes

The smallest networks of interest are those consisting of two nodes. They provide a good starting point, since the number of different networks is small enough to consider all. Furthermore, dynamics of two node networks can be studied through phase plane analysis. That enables a general analysis of two node networks. Dynamics of specific networks can be analysed using bifurcation theory, as explained in [8], and the MATLAB toolbox MatCont [9], which contains numerical methods for parameter continuation.

### 3.1 General analysis of two node networks

Before turning to specific examples, some general results can be obtained for networks consisting of two nodes. Let the general form of all two node networks be given by

$$
\left\{\begin{array}{l}
x_{1}^{\prime}=-x_{1}+S\left(f_{1}\left(x_{1}, x_{2}, p\right)\right)  \tag{5}\\
x_{2}^{\prime}=-x_{2}+S\left(f_{2}\left(x_{1}, x_{2}, p\right)\right),
\end{array}\right.
$$

where $p$ is a parameter and $S$ the logistic sigmoid as explained in 1 . Let A be the region trapped by the lines $x_{1}=1, x_{2}=1, x_{1}=0$ and $x_{2}=0$.

Note that in general, $0 \leq S\left(f_{1}\right), S\left(f_{2}\right) \leq 1$. In A, it also holds that $0 \leq x_{1}, x_{2} \leq 1$. Therefore, on the lines $x_{i}=1, i \in\{1,2\}$, the derivative $x_{i}^{\prime}$ is given by

$$
\begin{equation*}
x_{i}^{\prime}=-1+S\left(f_{i}\left(x_{1}, x_{2}, p\right)\right) \leq-1+1=0 \tag{6}
\end{equation*}
$$

On the lines $x_{i}=0$, it holds that

$$
\begin{equation*}
x_{i}^{\prime}=S\left(f_{i}\left(x_{1}, x_{2}, p\right)\right) \geq 0 . \tag{7}
\end{equation*}
$$

Hence, region A is invariant. It must therefore contain an $\omega$-limit set that is either an equilibrium, a periodic orbit or equilibria connected by orbits (Poincaré-Bendixson).

If there is a periodic orbit in A, the divergence of $S(f)$ should change sign within A. The divergence is given by

$$
\begin{equation*}
\nabla(S(f))=\frac{d S}{d f_{1}} \frac{d f_{1}}{d x_{1}}+\frac{d S}{d f_{2}} \frac{d f_{2}}{d x_{2}} . \tag{8}
\end{equation*}
$$

Note that since $S$ is the logistic function, its derivative is given by

$$
\begin{equation*}
\frac{d S}{d f}=k S(f)(1-S(f)) \geq 0 \quad \forall x_{1}, x_{2} \tag{9}
\end{equation*}
$$

In general, $f_{1}$ and $f_{2}$ are given by

$$
\left\{\begin{array}{l}
f_{1}=a_{0}+a_{1} x_{1}+a_{2} x_{2}  \tag{10}\\
f_{2}=b_{0}+b_{1} x_{1}+b_{2} x_{2},
\end{array}\right.
$$

where $a_{i}$ and $b_{i}$ are parameters. It follows that

$$
\left\{\begin{array}{l}
\frac{d f_{1}}{d x_{1}}=a_{1}  \tag{11}\\
\frac{d f_{2}}{d x_{2}}=b_{2} .
\end{array}\right.
$$

Hence, the system has no periodic orbits in region $A$ if $\operatorname{sign}\left(a_{1}\right)=\operatorname{sign}\left(b_{2}\right)$, that is, if both nodes have auto-activation, if both nodes have auto-inhibition, or if at most one of the nodes interacts with itself. In those cases, it follows that the system must have at least one equilibrium for $0 \leq x_{1}, x_{2} \leq 1$. It might contain a periodic orbit if $\operatorname{sign}\left(a_{1}\right)=$ $-\operatorname{sign}\left(b_{2}\right)$.

Note that if $f_{1}$ and $f_{2}$ are as in Equation (10), the system has an equilibrium at the origin if and only if $a_{0}=b_{0}=0$, that is, if and only if the nodes have no external input.

### 3.2 Analysis of all possible two node networks

Now we have some general information on the behaviour of two node networks, we can move closer to the main question. What networks share some of the dynamical behaviour of Kerkhofs' model? The key aspect there is the 'fight for dominance' of two imaginary factors. It implies that the dynamics on the networks should be multistable. More specifically, both factors should be able to 'win' and a 'win' for one factor should mean a 'loss' for the other. The outcome should be binary.

In terms of equilibria, a binary outcome implies the following. If one of the factors has convincing final activity, let's say larger than a half times $x 0(=0.25)$, then the activity of the other factor should end up close to zero. Multi-stability implies that the opposite should also occur. In other words, the system should have equilibria near $\left(x_{1}, x_{2}\right)=\left(\eta_{0}, 0\right)$ and $\left(x_{1}, x_{2}\right)=\left(0, \xi_{0}\right)$, where $0.25<\eta_{0}, \xi_{0} \leq 1$.

Let's investigate the existence of those equilibria in the most general network dynamics, given by system (12) below. Parameters $a_{0}$ and $b_{0}$ represent the base level of activation. Parameters $a_{2}\left(b_{1}\right)$ denote the strength and sign of the effect of $x_{2}$ on $x_{1}\left(x_{1}\right.$ on $\left.x_{2}\right)$. Note that taking $a_{2}$ or $b_{1}$ equal to zero would result in a reducible network, which is excluded from this study. Parameters $a_{1}\left(b_{2}\right)$ denote the strength and sign of $x_{1}\left(x_{2}\right)$ interacting with itself. The system is then as follows:

$$
\left\{\begin{array}{l}
x_{1}^{\prime}=-x_{1}+S\left(a_{0}+a_{1} x_{1}+a_{2} x_{2}\right)  \tag{12}\\
x_{2}^{\prime}=-x_{2}+S\left(b_{0}+b_{1} x_{1}+b_{2} x_{2}\right) \\
a_{0}, b_{0} \in[0,1], a_{1}, b_{2} \in[-1,1], a_{2}, b_{1} \in[-1,1]-\{0\}
\end{array}\right.
$$

To simplify matters, let's start with the special case $\eta_{0}=\xi_{0}=1$, often referred to as 'winner takes all' (WTA). Equilibria of the system above can be found by considering the nullclines:

$$
\left\{\begin{array}{l}
x_{1}=S\left(a_{0}+a_{1} x_{1}+a_{2} x_{2}\right)  \tag{13}\\
x_{2}=S\left(b_{0}+b_{1} x_{1}+b_{2} x_{2}\right)
\end{array}\right.
$$

If the system above has an equilibrium at $(1,0)$, it follows that

$$
\left\{\begin{array}{l}
S\left(a_{0}+a_{1}\right)=1  \tag{14}\\
S\left(b_{0}+b_{1}\right)=0
\end{array}\right.
$$

This result has two important implications. Firstly, $a_{1} \geq 0$, since $a_{0}+a_{1} \geq 1$ and $a_{0} \leq 1$. Secondly, $b_{0}+b_{1} \leq 0$ and $b_{0} \geq 0$ imply $b_{1}<0$. In other words, a WTA equilibrium can only be found in networks with at least one inhibitory connection between nodes. The winning node here is always an inhibited node. In addition, this winning, inhibited node does not inhibit itself.

As an example, networks with an activating connection in one direction and an inhibiting connection in the other can have a WTA equilibrium, as long as the inhibited node does not inhibit itself. Can that network, however, have two WTA equilibria? That is, can both nodes win? By symmetry of system (12), existence of an equilibrium at $(0,1)$ implies $b_{2} \geq 0$ and $a_{2}<0$. In other words, bistability in combination with a binary outcome only happens in networks with mutual inhibition, without auto-inhibition.

Suppose nodes in such a network only receive input (base level activation) if they do not activate themselves or receive activation from other nodes. Then $a_{1}=0$ if and only if $a_{0} \neq 0$ and $b_{2}=0$ if and only if $b_{0} \neq 0$. That limits the possibilities for the existence of the equilibrium $(0,1)$. The condition $a_{0}+a_{1} \geq 1$ now implies $a_{0}=1, a_{1}=0$ or $a_{0}=0, a_{1}=1$. In the same way, $b_{0}+b_{2} \geq 1$ now implies $b_{0}=1, b_{2}=0$ or $b_{0}=0, b_{2}=1$. That results in the networks in Figure 4 below.


Figure 4: All possible networks with mutual inhibition (up to topological equivalence), excluding those with auto-inhibition

Change in parameter might result in shift of location, or in disappearance. What happens? Let's zoom in on the network in Figure 4c. The conditions $a_{0} \leq a_{2}$ and $b_{0} \leq b_{1}$ then hold for $a_{2}=b_{1}=1$. Starting there, the picture shows that saddle-node and cusp bifurcations turn up for decrease in parameters. Continuation in MatCont shows a cusp point $(\mathrm{CP})$ at $(0.11,0.11,0.41,0.41)$ for $b_{1}=a_{2}=1$, for example. Cusp bifurcations can also be found when varying $a_{2}, b_{1}$ instead, where equilibria disappear for decreasing $a_{2}, b_{1}$, and when varying $a_{0}, a_{2}$. The diagram to the right in Figure 5 shows that the equilibria shift a bit, but not much. The WTA equilibria can both shift and disappear for certain parameters, but the win remains WTA for quite some time before disappearing.



Figure 5: Bifurcation analysis of the network in Figure 4 c with $a_{2}=b_{1}=1$. Left: Bifurcation diagram. Right: Following the position of an equilibrium for $b_{0}=1$.

The equilibria of network 4 a (without external input and $a 2=b 1=1$ ) behave similarly, except that there is no cusp bifurcation with respect to $a_{1}, b_{2}$. Instead, there are two separate saddle-node bifurcations. Also, $a_{0}=b_{0}=0$ is now a relevant scenario, in which this network always has an equilibrium at the origin. All this can be seen in Figure 6.


Figure 6: Nullclines of the system corresponding to the network in Figure 4a $\left(a_{2}=b_{1}=1, a_{0}=b_{0}=0\right)$ for $a_{1}=b_{2}=1$. Decreasing $b_{2}$ moves the line corresponding to $x_{2}^{\prime}=0$ to the left. Decreasing $a_{1}$ moves the line corresponding to $x_{1}^{\prime}=0$ downwards.

Decreasing $b_{2}$ shifts the line corresponding to $x_{2}^{\prime}=0$ to the left, without any deformation. Both lines meet at roughly $(0,0.9)$ when $b_{2} \approx 0.8$. In a similar way, the line corresponding to $x_{1}^{\prime}=0$ shifts downward for decreasing $a_{1}$, meeting the other nullcline at roughly $(0.9,0)$ for $a_{1} \approx 0.8$. Note that the equilibria stay at $(0,1),(1,0)$ for all (negative) $a_{2}, b_{1}$ (and $a_{0}=b_{0}=0$ ), since $a_{0}+a_{2} \leq 0, b_{0}+b_{1} \leq 0$ holds for all (negative) $a_{2}, b_{1}$.

What if instead of WTA wins, we are happy with $\left(\eta_{0}, 0\right)$ and $\left(0, \xi_{0}\right)$ as equilibria with $0<\eta_{0}, \xi_{0}<1$ ? This yields

$$
\left\{\begin{array}{l}
S\left(a_{0}+a_{1} \eta_{0}\right)=\eta_{0}  \tag{15}\\
S\left(b_{0}+b_{1} \eta_{0}\right)=0
\end{array}\right.
$$

The first equation implies $\eta_{0}=S\left(a_{0}+a_{1} \eta_{0}\right)$. For $a_{1}<0$, it has solutions for all $\eta_{0}$, depending on $a_{0}, a_{1}$. By taking $a_{0}=1, a_{1}=-\frac{1}{n-1}$ and $\eta_{0}=\frac{n-1}{n}, n>1, \eta_{0}$ can obtain many values between $\frac{1}{2}$ and 1 . Hence, $a_{0}$ can be varied such that $\eta_{0}$ can obtain any value between 0 and 1. This can also be observed in the plot in Figure 7.


Figure 7: $y=\eta_{0}$ and $y=S\left(a_{0}+a_{1} \eta_{0}\right)$ for $a_{0}=0,0.5,1$ from left to right. The sigmoid moves towards the right for increasing $a_{0}$. Note that increasing $a_{1}$ would steepen the sigmoid, resulting in an intersection for larger $\eta_{0}$.

For the second equation to hold, $b_{1}<0$, regardless of $\eta_{0}$. Again, by symmetry, bistability in combination with a binary outcome is then only met if in addition $a_{2}<0$. In other words, we still need mutual inhibition, but auto-inhibition is now allowed. However, bifurcation analysis as shown in Figure 8 shows that the auto-inhibition should not be too strong. If $a_{1}$ and $b_{2}$ become too negative, stable equilibria disappear in a saddle node or cusp bifurcation.


Figure 8: Bifurcation diagram of network 4 c with $a_{0}=b_{0}=1$ where parameters $a_{1}$ and $b_{2}$ are varied instead of being kept equal to zero.

We have so far seen two types of networks: those with inhibition to one side and activation to the other, and those with mutual inhibition. What is left, are the networks with mutual activation. Their dynamical behaviour can be predicted by sheer reasoning and for the sake of completeness will be described below.

The network with mutual activation and without self-inhibition, always has three equilibria: a stable equilibrium at $(0,0)$, a stable one at $(1,1)$ and a saddle. The network with mutual activation and one auto-inhibiting node is of a similar nature, although the autoinhibiting node might achieve a lower final activity. The network with two auto-inhibiting nodes might have an equilibrium at $\left(\eta_{0}, \xi_{0}\right), 0<\eta_{0}, \xi_{0}<1$, instead of at (1,1). If inhibition is strong enough, the equilibrium previously at $(1,1)$ might even be at $(0,0)$, coinciding with the off-equilibrium. If the nodes receive external input, however, the equilibrium at $(0,0)$ will vanish.

We have characterised networks by their equilibria, but what about possible periodic orbits? Those networks we would rather avoid. We saw before that periodic orbits can occur if one node has self-inhibition and the other self-activation. It turns out that such networks with mutual activation or mutual inhibition do not make likely candidates (see Appendix D for the reasoning). That leaves the two candidates in Figure 9.

(A)

(B)

Figure 9: Networks with possibility of periodic orbits

Simulation in the plane reveals that 9a has a periodic orbit if node 1 and node 2 act on different time scales, that is, if node 1 is slow compared to node 2 . This was modelled by
multiplying the differential equation for node 1 by $\epsilon=0.1$. The result is shown in Figure 10.

With the same separation of time scales, network 9 b has a periodic orbit if input is added to node 1.


Figure 10: Phase plane diagram of network 9a for parameters $a_{1}=0.6, a_{2}=b_{0}=$ $b_{1}=b 2=1$, time scaling constant $\epsilon=0.1$. The equilibrium is an unstable focus and the limit cycle is stable.

### 3.3 Summary of results: behaviour of two node networks

In the process of looking for bistable, binary outcome networks, we have come across three types of networks:

I - Mutual inhibition (A: WTA, B: regular)
II - inhibition-activation (A: without periodic orbits, B: with periodic orbits)
III - Mutual activation (A: full on-off, B: on-off with less than full activity in on-state).
Behaviour of the dynamics on those networks is summarised below.

## Type I - mutual inhibition

Networks with mutual inhibition are bi- or multistable for specific parameters, with binary outcome. The networks can lose equilibria through saddle-node bifurcations, where change in parameter causes a stable node equilibrium to merge with a saddle and both disappear.

All parameters involved in the system can cause a loss of equilibrium in this way. Networks with auto-activation and without external input lose a win equilibrium for decreasing auto-activation. More specifically, decrease in auto-activation on one node causes the win of that node to disappear. Note that the other win is left in place, just like the equilibrium at the origin.

Networks with external input cannot have an equilibrium at the origin. Changing the input on those networks can cause win equilibria to disappear, leaving a stable equilibrium with weak activity in all nodes. Networks with auto-inhibition also lose a win for a node if the auto-inhibition on that node becomes too strong.

All networks of this type are shown below in Figure 11 and 12.


Figure 11: A: All possible networks with mutual inhibition (up to topological equivalence), excluding those with auto-inhibition




Figure 12: B: All possible networks with mutual inhibition and a form of autoinhibition (up to topological equivalence)

## Type II- inhibition-activation

The set of inhibition-activation networks contains all networks with periodic orbits, namely the networks in Figure 14a and 14b. Apart from that, the networks behave like a combination of type I and III. They can be bistable but have no binary outcome. One of the equilibria can be a single win. Another possibility is activity in both nodes. If there is no input, there is an equilibrium at the origin.


Figure 13: Inhibition-activation pattern

(A)

(в)

Figure 14: Inhibition-activation networks with possibility of periodic orbits

## Type III - mutual activation

Networks with mutual activation behave like on-off switches. Networks without autoinhibition have stable equilibria at $(1,1)$ (on) and ( 0,0 ) (off). Networks with auto-inhibiting nodes might have an on-state with less activity. That activity might be unequally spread between nodes. In case of sufficient auto-inhibition of both nodes, the network might instead be permanently off. If nodes receive external input, however, the network is always weakly on.

## 4 Three nodes

So far, we have found three categories of two-node networks that might be regarded as motifs. What happens if we add a node? Three node networks might contain two node motifs and those might decide the behaviour. We might also find entirely new motifs consisting of three nodes. To find out, let's start by listing all interesting networks.

### 4.1 Finding all three node networks of interest

A list of relevant three node networks was obtained through the computer code discussed in Appendix C.1. When executed, the code creates a list of all topologically unique networks,
in the form of integers that uniquely correspond to adjacency matrices. In those matrices, inhibition is denoted by -1 , activation by 1 and no interaction by $0 .{ }^{1}$

To limit the number of resulting networks, certain matters were simplified. The notions of self activation and self inhibition were neglected and a base level of activation was later added to all nodes that are not activated by other nodes. Hence, criterion 2 ('every node needs an activation') requires an update. The new list of criteria for selecting interesting networks is as follows. Networks were deemed interesting if

- they do not contain messenger nodes, that is, nodes with one activating input and one activating or inhibiting output. Networks that do, can be recognised by an adjacency matrix where the $n^{t h}$ row consists of zeros and a single one, and the $n^{t h}$ column consists of zeros and a single nonzero input.
- all nodes in the network receive input, be it activation or inhibition. Nodes that do not receive input, can be replaced by a constant activation of the nodes they act on. Networks containing those non-receiving nodes can be recognised by zero rows in the adjacency matrix.
- they cannot be separated into smaller networks between which communication is one-directional. Networks that can, can be reduced and analysed by first considering the initiator part, calculating its equilibria and using those equilibria to calculate the equilibria of the receiving part. For three nodes, those reducible networks can be recognised by zero columns in the adjacency matrix.

These criteria led to sixty different three node networks. A full list can be found in Appendix A.

### 4.2 Methods of analysis

Analysis of the full list of networks was largely done numerically. To this extend, the dynamical details regarding those networks were filled in as follows. All nodes that did not receive activation from another node, received external input in the form of a parameter. The other parameters, representing the strength of interactions, were assumed equal and set to 1 . From there, systematic numerical analysis was conducted.

Like with analysis of two node networks, the aspects of interest were the location and stability of equilibria, and the occurrence of periodic orbits. The former was analysed by performing a thousand Newton iterations with random initial conditions and a fixed set of different input parameters, gathering location and stability of (nearly) all equilibria. The results were backed up by another thousand Newton iterations with random input parameters, giving only the possible number of equilibria as output.

Periodic orbits are a bit harder to detect. Networks with the suspicion of a periodic orbit were analysed in MatCont. For example, some networks had a node switching to a saddle with parameter change, without change in the number of equilibria. Such behaviour might indicate a saddle-node bifurcation of periodic orbits. That is, a repelling and an attracting periodic orbit might coalesce, causing the centre node to lose stability in the direction of the periodic orbit. Other suspicious networks are those that directly or indirectly contain a two node motif of case II-A (inhibition-activation, as in Figure 14a).

The direct or indirect occurrence of two node motifs in networks was checked as follows. Let $\mathrm{a}, \mathrm{b}$ and c be nodes.

[^0]- For every activation from $a$ to $b$ followed by an activation from $b$ to $c$, an indirect activation was denoted from a to c.
- For every activation from a to b followed by an activation from b to a , an indirect self-activation was denoted for a.
- For every activation from a to $b$ followed by an inhibition from $b$ to $c$, an indirect inhibition was denoted from a to c.
- For every activation from a to $b$ followed by a inhibition from $b$ to $a$, an indirect self-inhibition was denoted for a.
- For every inhibition from a to $b$ followed by an activation from $b$ to $c$, an indirect inhibition was denoted from a to c.
- For every inhibition from a to b followed by an activation from b to a , an indirect self-inhibition was denoted for a. ${ }^{2}$

After being subjected to the list of steps, the networks were scanned for motifs of mutual inhibition (type I) and inhibition-activation (type II). Some networks contained both. If those networks directly contained one of the motifs, rather than indirectly, said motif was taken as leading.

All acquired results were processed as follows. Networks were sorted based on their maximum number of equilibria for varying input. If only one equilibrium was found, the network was denoted as unistable. Next, the equilibrium was classified as either a WTAwin, a regular win, activation everywhere, or no activation at all. If two or more equilibria were found, the network was denoted as bistable or multistable. Based on the location and stability of the equilibria, the network was classified as either all-on-all-off (type III in two nodes), a win and a bit of activity everywhere (type II in two nodes), two WTA-wins, or two regular wins (type I in two nodes). If a saddle and a node equilibrium appeared or disappeared in a network for varying input, this was denoted as a saddle-node bifurcation. Other denoted aspects were occurrence of a periodic orbit and occurrence of two node motifs.

### 4.3 Results

For two nodes, we recognised three different types of bistability: with a binary outcome (type I), in the form of an on-off switch (type III), and a mix of the two: one win and one off or weakly on equilibrium (type II). Through bifurcations, bistable systems became unistable, sometimes in combination with a periodic orbit. For three nodes, similar types of behaviour can be found. What follows is a description of the two-node motifs and occurrence of saddle-node bifurcations and periodic orbits per category of networks, categorised based on their number and type of equilibria as described in the previous section. Behaviour per network and lists of networks per category can be found in Appendix B.

Networks are referred to by their number. A full list of networks and their number can be found in Appendix A.

## Bi- or multistability with binary outcome

[^1]All bistable three node networks with binary outcome contain mutual inhibition and the multistable networks contain it multiple times. WTA networks do not contain direct inhibition-activation. All other bistable binary outcome networks do, like 161, for example.

Some of the networks feature a saddle-node bifurcation. Network 184 even features two saddle-node bifurcations for increasing input; it starts with an


161 equilibrium at the origin that splits into two win equilibria, but one of those disappears as input increases further. Networks that do not feature a saddlenode bifurcation, have equilibria that shift towards a more convincing win. Incidentally, those networks are the only networks containing a mutual activation motif.

## Bistability as an on-off switch

Only two networks behave like a true on-off switch, namely 121 and 364. Both networks only contain mutual activation, so no mutual inhibition or inhibitionactivation.

## Bistability with win and weak on or off state

The case of 'bistability with win and off state' perhaps requires clarification. Two stable equilibria were found, where one node is off in both equilibria and the other nodes are either on or off (equilibria at $(1,0,1)$ and ( $0,0,0$ ), for example).

It turns out that none of those networks had external input. Two of the bistable win-offs contain the mutual inhibition motif (149 and 457). All networks contain inhibition-activation and mutual activation.


457


157


152

## Unistability - always off; weakly on

Networks that are always off, all contain the inhibition-activation and the mutual activation motif, like network 122 .

The group of unistable networks with activity in all nodes (weakly on) all contain inhibition-activation motifs except for 182, which does not contain any motifs. Network 220 also contains indirect mutual inhibition.


Network 182 deserves some extra attention. It exhibits a saddle-node bifurcation without extra equilibria. When inspected in MatCont, this turns out to be a saddle-node bifurcation of periodic orbits. Hence, next to mutual inhibition, mutual activation and inhibition-activation, we seem to have found a new motif.

Since the network reminds one of a merry-go-round, it is from now on referred to as the merry-go-round motif. It features in networks 185, 188, 212, $215,239,242,455,458,485$ and 728 . What this motif does exactly and how it works requires further investigation and is outside the scope of this study.


182
Network 215 is hard to place. With equilibria at $(.1, .2, .5)$ and $(1,0,0)$ for parameters (. 8,1 ), it might belong to the 'win-weakly on' category, but one might argue that 0.1 is close enough to zero compared to 0.5 . Hence, it is also close to a binary outcome. One of the wins appears after a saddle-node bifurcation. In addition, it has a saddle-node bifurcation without extra equilibria. When inspected, it has a saddle-node bifurcation of periodic orbits.

Motif-wise, it contains mutual inhibition, inhibition-activation and the new


215 merry-go-round motif.

## 5 Discussion

The results in the previous section seem to suggest that motifs and network behaviour are related. To see how, the roles of motifs were analysed per category, followed by a summary per motif.

### 5.1 Bi- or multistability with binary outcome

All networks in this category contain a form of mutual inhibition, so for these networks the motif seems to determine the dynamical behaviour. In other words, mutual inhibition seems to play an important role in multistability with a binary outcome.

Inhibition also seems to play a role in the occurrence of saddle-node bifurcations. Network 184 even features two. Its mutual inhibition motif looks like mutual inhibition with auto-inhibition on one node. This type is not excessively discussed in Section 3. However, when looking at the network structure one may reason as follows.

The strength of inhibition on node 1 depends on the external input in node 2. Hence, decreasing input in node 2 implies decreasing the strength of inhibition in node 1 . If auto-inhibition is too strong, node 1 cannot win, but it might be able to win if inhibition from 2 is less strong. Then as input on node 2 increases, node 1 loses its ability to win whereas node 2 gains ability to win.

The characteristic saddle-node bifurcation of mutual inhibition in two nodes was not present in the three node networks with mutual activation. Incidentally, those are also the networks with only one


Network 184 input parameter. They might be compared to the network in Figure 4b, which has not been as thoroughly analysed as the other mutual inhibition networks consisting of two nodes. It might be that equilibria of that network shift there as well, rather than undergoing bifurcation. The bifurcation point might just never be reached; equilibria that do appear
in a saddle node bifurcation, also continuously move to a state of increased activity after appearing.

What, however, is the biological relevance of this equilibrium shift? Is it an alternative to Ferrell's notion of a saddle-node bifurcation, or sign of a set of networks that bares no relevance? If such sudden parameter changes can happen to an actual cell and equilibria shift rather than disappear, cell differentiation does not lead to the loss of a stable state, in dynamical terms. However, if that stable state suddenly implies apoptosis and the position of the saddle has also changed, then shifting of the equilibrium practically has the same effect as disappearing.

### 5.2 Bistability as an on-off switch

All on-off switches contain mutual activation, and activation only. The on-state then comes as no surprise. The off-state is a result of a lack of input on the nodes. Just like in two node systems, there exists an equilibrium at the origin if and only if the input is zero. This fact follows from the most general system of three nodes,

$$
\left\{\begin{array}{l}
x_{1}^{\prime}=-x_{1}+S\left(a_{0}+a_{1} x_{1}+a_{2} x_{2}+a_{3} x_{3}\right)  \tag{16}\\
x_{2}^{\prime}=-x_{2}+S\left(b_{0}+b_{1} x_{1}+b_{2} x_{2}+b_{3} x_{3}\right) \\
x_{3}^{\prime}=-x_{3}+S\left(c_{0}+c_{1} x_{1}+c_{2} x_{2}+c_{3} x_{3}\right), a_{0}, b_{0}, c_{0} \in[0,1]
\end{array}\right.
$$

where it is easily seen that $x_{1}^{\prime}=x_{2}^{\prime}=x_{3}^{\prime}=0$ can only hold for $\left(x_{1}, x_{2}, x_{3}\right)=(0,0,0)$ if $a_{0}=b_{0}=c_{0}=0$.

### 5.3 Bistability with win and weak on or off state

All of the networks with a win and a weak on or off state have inhibition-activation and mutual activation. Inhibition-activation was to be expected, since the networks with input have a weak on state and the networks without input have an off-state, and this is how the two-node network behaves. Mutual activation between two nodes seems responsible for having them win and lose together.

Two of the bistable win-offs contain the mutual inhibition motif (149 and 457). Mutual inhibition here seems responsible for the single win. A win for the node on the other side of the mutual inhibition might be impossible because of a lack of activation of that node.

Take network 149. It represents a fight between node 2 and 3 . Node 3 might be said to have auto-activation via node 1, but node 2 does not have auto-activation, only activation via node 1. Reasoning from the two-node network in Figure 4a, the activation via node 1 on node 2 might simply not be big enough.

### 5.4 Unistability as a win

We saw that most of the networks in this category contain mutual


Network 149 inhibition. Many of those have a form of extra inhibition in the network, like 152. It might be that those networks are bistable with binary win if that extra inhibition is taken small enough. In that case, unistability is the result of a saddle-node bifurcation, with respect to parameters corresponding to the strength of interactions.

Most of the networks also contain the inhibition-activation motif. It might therefore also be seen as dominance of the inhibition-activation motif, although the exact behaviour of this motif is not as thoroughly studied in Section 3.

### 5.5 Unistability - always off; weakly on

Networks that are always off or weakly on all contain the inhibition-activation and the mutual activation motif. What is the role or effect of inhibition-activation here? It appears that the inhibition-activation pattern facilitates indirect auto-inhibition; the networks without external input are always off. The ones with external input are weakly on.

Network 220 also contains indirect mutual inhibition. It may be such that the interaction parameters fall outside the cusp wedge of the bifurcation diagram, so that only one equilibrium remains, with activity everywhere. Change in $a_{2}$ and $b_{1}$ in the network in Figure 4 c also resulted in cusp bifurcation for $a_{0}=b_{0}=1$, after all.

### 5.6 How do motifs relate to dynamical behaviour?

In the analysis of all categories above, we have seen different effects of two-node motifs in three-node networks. What follows is a summary per motif.

Mutual inhibition between nodes seems responsible for the networks with binary outcome. One of the wins might disappear in a saddle-node bifurcation. It is also possible that one of the win equilibria does not exist, perhaps because of the existence of a saddle-node bifurcation with respect to strength of interactions. If the network receives no external input, this results in the win-off situation, since the origin is an equilibrium for all networks without input. If there is input, the network falls into the single-win category.

Mutual activation between nodes seems responsible for having nodes win and lose together.

The effect of inhibition-activation seems mostly dependent on other motifs. Sometimes the motif acts as auto-inhibition on a node. To illustrate, WTA networks with mutual inhibition do not contain direct inhibition-activation. Binary outcome networks that do, all have wins with less activity, as is the case for two-node networks with auto-inhibition. In the presence of mutual activation, without mutual inhibition, inhibition-activation sometimes forces a network to always be off.

At other times the inhibition-activation motif facilitates a single win, or a weak onequilibrium (the off equilibrium is there if and only if there is no input). Also, periodic orbits might arise without separation of time scales.

Another motif linked to periodic orbits is the merry-go-round. However, not all of the networks found to contain periodic orbits in combination with inhibition-activation contain the merry-go-round motif, so the existence of periodic orbits in those networks can be contributed to the inhibition-activation motifs. Similarly, the existence of periodic orbits in networks with the merry-go-round motif can be contributed to the merry-go-round motif.

## 6 Reflection

Some aspects of this study require extra attention.
Firstly, certain equilibria might be missing, because some equilibria have a Jacobian equal to zero, in which case Newton iterations cannot be used and one should resort to continuation of the equilibrium with a change of variables, like arc length continuation. In this case the missing equilibria must be saddles, so this does not cause a bistable system to pass by as a unistable system.

Secondly, not all networks have been checked on occurrence of periodic orbits. Networks without a saddle-node bifurcation of periodic orbits and without the specific inhibition-
activation motif might have periodic orbits, too. However, periodic orbits are not the focus of this study; they are assumed to be of little relevance to bistability on GRNs. Biological relevance of periodic orbits lies in periodic systems like day-night rhythm, for example. Another application of periodic orbits in GRNs has been found in a phenomenon called 'bursting' [10].

More importantly, not all parameters have been varied to assess robustness of the outcomes. The strength of interactions is set equal, but this might of course be varied to yield different results, and in biology this might frequently be the case. Unistable systems might then become multistable and have a binary outcome as we saw in some of the networks discussed above. Varying the parameters determining the sigmoid, that is, $k$ and $x_{0}$, shifts the location of the equilibria. That might lead to wins turning into WTA wins. The effect of the break-down factor, set to 1 in this study, has not been assessed.

Furthermore, it might be logical to add other parameters. Nodes that get activation from other nodes, could still be given external input. External input was seen as a constant factor, left out of the GRN for the sake of simplicity. In that case there is no reason to assume that otherwise activated nodes do not receive input.

Another logical next step is to extend the analysis to networks of more nodes; the question arises how the number of different motifs increases with the number of nodes, and how much of the behaviour of a smaller motif pops out in the behaviour of a larger network. The larger networks might have new motifs, like three-node networks have the merry-go-round that two-node networks do not have. It would be hard to follow the same procedure, however, as the number of unique networks explodes with the number of nodes. Computing all unique four-node networks already takes more than half a day, even if only interesting four-node networks are considered.

## 7 Conclusion

So far we have found out about the behaviour of three types of two-node networks. Mutual inhibition leads to bistability and saddle-node bifurcations, mutual activation leads to onoff switches and inhibition-activation might lead to periodic orbits. As motifs in three-node networks, they seem to induce similar behaviour. Three-node networks also know a fourth motif with characteristic behaviour of periodic orbits: the merry-go-round.

It seems unlikely that periodic orbits play a role in bistability in cell differentiation. On-off switches might appear in a bistable system but are not the cause of bistability with a binary outcome. The answer appears to lie with mutual inhibition.

However, new motifs that can also be linked to bistability, might be found in networks with a larger number of nodes. Furthermore, the question arises how much of the behaviour of a smaller motif pops out in the behaviour of a larger network.

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A Networks on number


103
107
121

122

125



238

224
229




392
395

458
476


484
485


728

## B Full results 3 node networks

Direct occurrence of motifs is denoted by ' $x$ '. Indirect occurrence of motifs is denoted in by ' $(\mathrm{x})^{\prime}$. Results are listed in the same order as they are discussed in Section 4.3.

Table 1: Multistable

| Integer | Saddle-Node Bifurcation | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 392 | - | x | $(\mathrm{x})$ | x |
| 728 | x | x | - | - |

TABLE 2: Bistable - binary outcome WTA

| Integer | Saddle-Node Bifurcation | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 104 | - | $(x)$ | - | x |
| 158 | - | x | - | x |
| 187 | x | x | - | - |
| 205 | - | x | $(\mathrm{x})$ | x |
| 214 | - | x | - | x |
| 224 | x | x | - | - |
| 233 | x | x | - | - |
| 242 | x | x | - | x |
| 476 | - | x | - |  |

TABLE 3: Bistable - binary outcome

| Integer | Saddle-Node Bifurcation | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 161 | x | x | x | - |
| 184 | x | $(\mathrm{x})$ | x | - |
| 211 | - | $(\mathrm{x})$ | x | x |
| 230 | x | x | x | - |
| 395 | - | x | x | x |
| 404 | x | x | x | - |
| 484 | x | x | x | - |
| 485 | x | x | x | - |

Table 4: Bistable - On-Off

| Integer | Saddle-Node Bifurcation | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 121 | - | - | - | x |
| 364 | - | - | - | x |

TABLE 5: Bistable - one win, one off equilibrium

| Integer | Saddle-Node Bifurcation | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 124 | - | - | x | x |
| 130 | - | - | $(\mathrm{x})$ | x |
| 131 | - | x | x |  |
| 148 | - | x | x |  |
| 149 | - | $\mathrm{x})$ | x |  |
| 365 | - | x | x |  |
| 374 | - | - | x | x |
| 457 |  | x | x |  |

TABLE 6: Bistable - one win, one equilibrium with activity in all nodes

| Integer | Saddle- <br> Node- <br> Bifurcation | Periodic- <br> Orbit | Mutual- <br> Inhibition | Inhibition- <br> Activation | Mutual- <br> Activation |
| :--- | :--- | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |  |
| 157 | - | x | - | $\mathrm{x}($ IIA $)$ | x |
| 103 | x | - | - | $(\mathrm{x})$ | x |
| 202 | x | - | - | x | x |
| 368 | - | x | - | $\mathrm{x}($ IIA $)$ | x |

Table 7: Unistable - WTA win

| Integer | Periodic Orbit | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 107 | - | $(x)$ | x | - |
| 188 | - | x | - | - |
| 203 | - | x | - |  |
| 206 | - | x | - |  |
| $221-$ | x | x | - |  |
| 232 | - | x | - |  |
| 241 | x | x | - |  |

TABLE 8: Unistable - win

| Integer | Periodic Orbit | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 134 | - | $(x)$ | x | - |
| 152 | - | x | x | - |
| 212 | - | - | x | - |
| 229 | - | x | - |  |
| 238 | - | x | - |  |
| 239 | - | $\mathrm{x})$ | x | - |
| 377 |  | $\mathrm{x})$ | x | - |
| 458 |  | x |  | - |

TABLE 9: Unistable - no activity

| Integer | Periodic Orbit | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 122 | - | - | x | x |
| 133 | - | - | x | x |
| 151 | - | - | x | $(\mathrm{x})$ |
| 376 | - | - | x | $\mathrm{x})$ |
| 455 | - | - | x |  |

Table 10: Unistable - activity in all nodes

| Integer | Periodic Orbit | Mutual Inhibition | Inhibition-Activation | Mutual Activation |
| ---: | :--- | :--- | :--- | :--- |
| 0 | - | - | - | - |
| 106 | - | - | x | - |
| 125 | - | - | x | - |
| 160 | - | - | x | - |
| 182 | x | - | - |  |
| 185 | - | - | x | - |
| 220 | - | $(\mathrm{x})$ | x | - |

## C Code

## C. 1 Constructing a list of interesting three node networks

The following code was used to construct a list of all possible adjacency matrices of three node networks, with inhibiting and activating interactions, without considering base level of activation, self activation or self inhibition. This was done by uniquely mapping integers to a 3 -by- 3 matrix consisting of the integers 0,1 and 2 , representing no interaction, activation and inhibition. That mapping consists of converting base 10 integers to base 3 integers. To illustrate, let x be a base 10 integer smaller than $3^{6}$. Then

$$
x=\left(\begin{array}{lllll}
a_{5} & a_{4} & \ldots & a_{1} & a_{0}
\end{array}\right)\left(\begin{array}{c}
3^{5}  \tag{17}\\
3^{4} \\
\ldots \\
3^{1} \\
3^{0}
\end{array}\right), a_{i} \in\{0,1,2\} .
$$

Next, a 3-by-3 matrix was filled by the digits of those base 3 integers, leaving diagonal entries 0 . In this fashion the following matrix was obtained:

$$
\left(\begin{array}{ccc}
0 & a_{5} & a_{4} \\
a_{2} & 0 & a_{3} \\
a_{1} & a_{0} & 0
\end{array}\right) .
$$

From there, integers corresponding to topological equivalent matrices of other integers were removed from the list, to create a list of all possible unique 3 -node networks.

```
from numpy import identity, array, zeros, triu_indices,
    tril_indices, linalg, sign
import numpy as np
from itertools import permutations
b = 3 # number of options for interaction between nodes: none:0,
    activation:1, inhibition:2
def int2vec(x, n):
    """map integer x to n(n-1) vector [array] with non-diagonal
        entries,
    through computing the binary representation: divide x by
        powers of
    b, add the remainder to the vector (backwards)
    Test
    >> int2vec(5,3)
    array ([0, 0, 0, 1, 0, 1])
    """
    return array([(x // b ** i) % b for i in range(n * (n - 1))])
        [::-1]
```

def vec 2 int $(v, n)$ :
"""map $n(n-1)$-vector (adjacency-matrix entries) to an integer
Inverse
function of int2vec (). Idea: multiply the remainder with
powers of $b$.
Test
$\gg \mathrm{nn}=3$
$\ggg \operatorname{vec} 2$ int $(\operatorname{array}([0,0,0,1,0,1]), n n)$
5
$\ggg$ for $m$ in range $(2 * *(\mathrm{n} *(\mathrm{n}-1)))$ :
... assert vec2int (int2vec $(m, n), n)=m$
$\# \ldots \quad \operatorname{assert} \operatorname{vec} 2 \operatorname{int}(\operatorname{int} 2 \operatorname{vec} 2(m, n), n)=m$
" " "
$\mathrm{x}=0$
$\mathrm{v}=\mathrm{v}[::-1]$
for $i$ in range $(n *(n-1))$ :
$\mathrm{x}+=\mathrm{v}[\mathrm{i}] *(\mathrm{~b} * * \mathrm{i})$
return $x$
def int2mat2 (x, $n$ ):
" " "map integer $x$ to $n-b y-n$ adjacency matrix: diagonal-method.
Obtains
$n(n-1)$-vector, fill upper part of matrix with the first $n(n$
-1)/2 entries,
and lower triangular part with remainder.
Test
$\ggg$ int2vec $(5,3)$
$\operatorname{array}([0,0,0,1,0,1])$
$\gg \operatorname{int} 2$ mat2 $(5,3)$
array ([[ 0., 0., 0.],
$[1 ., 0 ., 0$.$] ,$
$[$ 0., 1., 0.]])
" I "
$\mathrm{v}=\operatorname{int} 2 \operatorname{vec}(\mathrm{x}, \mathrm{n})$
$\mathrm{am}=\operatorname{zeros}(\operatorname{shape}=(\mathrm{n}, \mathrm{n}))$
indur, induc $=$ triu_indices $(\mathrm{n}, ~ 1)$
indlr, indlc $=$ tril_indices $(n,-1)$
for $i$ in range (int $(\mathrm{n} *(\mathrm{n}-1) / 2))$ :
$\operatorname{am}[(\operatorname{indur}[i], \operatorname{induc}[i])]=\operatorname{int}(\mathrm{v}[\mathrm{i}])$
$\operatorname{am}[(\operatorname{indlr}[\mathrm{i}], \operatorname{indlc}[\mathrm{i}])]=\operatorname{int}(\mathrm{v}[\mathrm{i}+\operatorname{int}(\mathrm{n} *(\mathrm{n}-1) / 2)$
])
return am

```
def mat2vec(M, n):
    """map n-by-n adjacency matrix to n(n-1)-vector. Inverse of
        int2mat2.
    For all rows, the non-diagonal entries are placed in the
        vector, starting
    from the right-side of the diagonal.
    " " "
    y = zeros(n* (n - 1))
    for i in range(n):
        v}=\textrm{M}[\textrm{i}][0:
        z = zeros(n - 1)
        z[0:n-i - 1] = v[i + 1:n]
        z[n-i - 1:] = v[0:i]
        y[i * (n-1):(i + 1) * (n-1)] = z
    return y
def matcomp(x1, x2, n):
    """compare whether two integers correspond to equivalent
        adjacency-matrices.
    Map x1 to adjacency matrix, construct all possible
        permutations (reindexing
    of the nodes while leaving the topology intact), map these
        matrices to an
    integer and check whether it corresponds to x2. Prints '
        equivalent, if the
    matrices are equivalent and nothing if they are not.
    >>> matcomp (4, 8, 3)
    equivalent
    >> matcomp (4,7,3)
    " " "
    A= int2mat2(x1, n)
    M=range(n)
    Q = zeros(shape=(n, n))
    for q in permutations(M) :
        for i in range(n):
            for j in range(n):
                    Q[i][j] = A[q[i ]][q[j]]
        var = int(vec 2int(mat2vec(Q, n), n))
        if var = x2:
            print('equivalent')
```

```
def mateq(x1, n):
    """constructs a set of all equivalent adjacency-matrices for
        an integer.
    Map the integer to its adjacency matrix, compute all possible
        permutations
    (reindexing the nodes while leaving the topology intact), map
        equivalent
    matrices to integers and construct a set. Set does not give a
        sorted list.
    >>> mateq (4,3)
    set([32, 1, 2, 4, 8, 16])
    >>> mateq (5,3)
    set([34, 5, 40, 10, 17, 20])
    " " "
    A = int2mat2(x1, n)
    M = range(n)
    Q = zeros(shape=(n, n))
    eq = []
    for q in permutations(M):
        for i in range(n):
            for j in range(n):
                    Q[i][j] = A[q[i]][q[j]]
        var = int(vec2int(mat2vec(Q, n), n))
        eq.append(var)
    return set(eq)
def uniquelist(n):
    """construct a list of the number of unique integers for
        constructing a
    network with n nodes. Use mateq-function to obtain all the
        possible
    equivalence-permutations of an integer. Start at 0 and
        iterate this
    process for all 2^(n(n-1)) options. Add an integer to the
        list if it does
    not occur in any of the permutations-set of its previous
        added members, and if it obeys the criteria for an
    interesting network."""
    a = set([])
    out = set([])
    for i in range(b**(n*(n-2)+1), b**(n*(n-1))):
        A = int2mat2(i, n)
        B = A.transpose()
        if (sum(A[0]) = 1 and sum(array (B[0], bool)) =1) or i
```

```
            % b**(n-1)=0:
            out.add(i)
    for i in range(b**(n*(n-2)+1), b**(n*(n-1))):
        A = int2mat2(i, n)
        B = A.transpose()
        if (\operatorname{sum}(\textrm{A}[0])=1 and sum(array (B[0], bool)) =1) or i
            % b**(n-1)=0:
                continue
    v = mateq(i, n)
    if len(v & a) = 0 and len(v & out) = 0:
                a.add(i )
    return a
def weed(network_list,n):
    """ Removes all networks from a list that are deemed
        uninteresting but passed through uniquelist.
    Iterates over all networks in the list and checks if they
        should be kept or removed. All networks that should be
    kept are returned in a new list."""
    weeded_list = list()
    for m in network_list:
        weeded = False
        A= int2mat2(m, n)
        B = A.transpose()
        for row in A:
            if sum(row) = 0:
                weeded = True
                break
        for column in B:
            if sum(column) = 0:
                weeded = True
                break
            if not weeded:
                weeded_list.append(m)
    return weeded_list
n}=
nodes3 = weed(uniquelist(n),n)
nodes3.sort()
with open("ThreeNodeNetworks.txt", "w") as f:
    f.write(" [")
    f.write(str (nodes3[0]))
    for network in nodes3[1:]:
        f.write(", " + str(network))
    f.write("] ")
```


## D Two node networks with periodic orbits

This section contains elaborations for the interested reader on some of the aspects mentioned in Section 3 that were not deemed relevant enough for the article.

We saw before that periodic orbits can occur if one node has auto-inhibition and the other auto-activation. That leaves the four candidates in Figure 9.

(A) Mutual inhibition

(c) Inhibition-activation

(в) Mutual activation

(D) Inhibition-activation

Figure 27: Networks with possible periodic orbits
What we need for periodic orbits is pared oscillations in activity of node 1 and 2. Candidate 27 a is therefore not likely to have a periodic orbit. High activity in node 2 might result in a decrease of activity in that same node, but has no possibility to increase after that; inhibition does not become any less, and input is constant. Moreover, decrease of activity in node 2 only leads to increase in node 1 , and activity in node 1 can only decrease if node 2 increases again, which cannot happen. More activity in node 1 leads to inhibition of node 2 and therefore less activity in node 2. That leads to more activation of node 1 . In other words, node 1 wins. Similarly, more activity in node 2 leads to inhibition of node 1 and therefore less inhibition of node 2 . In other words, node 2 wins.

Candidate 27b cannot have a periodic orbit either. Activity of node 1 always increases and never decreases.

The networks in 27c and 27d, however, can have pared oscillations as described, provided the nodes act on different time scales. If activity in node 2 in 27 c decreases, activity in node 1 decreases, so that activity in node 2 can increase again. In network 27d, increase in activity in node 2 implies increase in node 1 , which implies decrease in node 2 .


[^0]:    ${ }^{1}$ Denoting the three options by 0,1 and 2 is perhaps more intuitive with regard to the notation of base 3 integers, but note that $2 \bmod 3=-1$.

[^1]:    ${ }^{2}$ The last two cases might seem redundant, because an inhibited note will never activate any other node if the former starts at zero activity. Note, however, that those inhibited nodes receive external input if they are not activated by other nodes. Hence, activity in the inhibited nodes can increase, so that they activate other nodes, anyway.

