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Generic bifurcations in the dynamics of biochemical networks

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Abstract— **Complex behavior in the dynamics of biochemical reaction networks is commonly governed by bifurcations of equilibria and limit cycles. In this tutorial, we discuss two frequent types of bifurcations, the saddle-node and the Hopf bifurcation, and their relation to bistability and oscillations in biochemical networks, respectively. We also present examples from molecular biology where the dynamical behavior of specific networks is defined by these bifurcations.**

I. INTRODUCTION

Most dynamical processes within living cells are based on biochemical reactions. Networks of biochemical reactions govern intracellular dynamics over various regimes, including metabolism, signal transduction, and gene expression. In signal transduction and gene regulation networks, the biological functionality is often tightly connected to the qualitative dynamical behavior of the network. Recent studies have identified basic motifs contributing to a certain biological process by displaying specific dynamical properties, for example switching behavior or oscillations [1], [2].

The motifs as basic building blocks of intracellular networks are embedded in a larger intracellular context, and also depend on intrinsic conditions. Accordingly, the mathematical models of the corresponding dynamics universally contain several parameters, representing either extrinsic or intrinsic conditions which are subject to environmental, genetic, or other variety. In such parametrized models, the dynamical properties are characterized by bifurcations, i.e. variations in the qualitative behavior occuring under changes in parameter values [3]. Thus, bifurcation analysis is an important tool for understanding dynamical properties related to biological functionality of biochemical networks.

The intention of this paper is to provide a tutorial about the basic mechanisms behind biochemical switches and oscillators from a bifurcation analysis perspective. Thereby, we discuss specifically the saddle-node bifurcation and how biochemical switches are based on this bifurcation type, as well as the Hopf bifurcation and its relation to biochemical oscillators.

The paper is structured as follows. The model classes to be studied, namely biochemical and regulatory networks, are presented in Section II. Section III describes the saddle-node bifurcation and its relation to biochemical and genetic switches. The Hopf bifurcation and its occurence in biochemical oscillators is presented in Section IV.

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II. MODELS OF BIOCHEMICAL NETWORKS

A *biochemical network model* (BN) describes interactions between different cellular components at a molecular level. As these interactions are usually of chemical nature, such as complex formation, degradation by proteases, or chemical modification of proteins, BNs are often based on chemical reaction kinetics. Dimerization of a protein P is for example described by a reversible reaction $2P \leftrightharpoons P_2$. A convenient representation of a set of chemical reactions with n species and r reactions is given by the ordinary differential equation (ODE) system

$$\dot{x} = Sv(x,\mu),\ x \in \mathbb{R}^n_+, \mu \in \mathbb{R}^q, S \in \mathbb{R}^{n\times r}, v \in \mathbb{R}^r, \qquad (1)$$

where x is the concentration vector of all species in the reaction system, and μ is a parameter vector. The stoichiometric matrix S contains the number of molecules of each species that enter the reactions. The reaction rates, which are usually described by mass action kinetics, are collected in the flux vector v.

Motivated by a huge amount of work on gene regulatory networks that describe regulation of gene expression via binding of transcription factors to the DNA, a model class called *regulatory network models* (RN) has intensively been investigated. Activation of transcription via binding of a transcription factor T to a specific binding site B in the promoter region of the regulated gene results in a complex C which enables the polymerase P to initiate transcription, and can for example be described by the reactions

$$T + B \quad \rightleftharpoons \quad C \qquad (2)$$

$$C + P + \text{ribonucleotides} \quad \rightarrow \quad C + \text{mRNA} + P. \qquad (3)$$

Since this binding event happens on a much faster time scale than gene expression, a quasi-steady state approximation leads to a functional relation between the transcription rate of the regulated gene and the transcription factor concentration, described by a Michaelis-Menten or Hill-equation (for more details see [1]). Formally, a RN is defined as

$$\dot{x} = f(x,\mu),\ x \in \mathbb{R}^n_+, \mu \in \mathbb{R}^q, f \in \mathcal{C}^1 \qquad (4)$$

with Jacobian matrix $\mathcal{J}_f(x)$ that has constant signs on its off-diagonal elements, indicating activating or inhibiting regulation. Some BNs also fulfill these monotonicity conditions, and methodology for RNs can as well be applied to them.

Graphical approaches for both model classes are surveyed in [4]. A RN can be illustrated by its *interaction graph* $G(V,E)$, that is, a directed graph, with vertex set V corresponding to species, and sign-labeled edges E. (Semi)paths and (semi)circuits in this graph are defined in the usual way

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[5], and signs of those are given by the product of signs of their edges. The interaction graphs will be the important representation for our purposes. We furthermore assume that the systems are stable in the sense that there exists a bounded trapping region in the state space, i.e. a forward invariant region that is eventually entered by all trajectories. This is for example obtained by introducing first-order degradation terms for each component.

The following analysis considers complex dynamic behavior emerging from the generic codimension-1 bifurcations of fixed points in BN and RN models, saddle-node and Hopf bifurcations.

III. THE SADDLE-NODE BIFURCATION AND BIOCHEMICAL SWITCHES

A. The saddle-node bifurcation

The saddle-node bifurcation (SNB) is a bifurcation where two steady states of the system (1) collapse and thereby disappear from the dynamics. The normal form of the SNB is given by the scalar ODE

$$\dot{x} = \mu - x^2, \tag{5}$$

where $\mu \in \mathbb{R}$ is a variable parameter [3]. Solving for the steady state of (5) yields

$$\bar{x} = \pm\sqrt{\mu}. \tag{6}$$

Thus the number of resulting steady states depends on the value of the parameter μ: for $\mu < 0$, there is no steady state, for $\mu = 0$, there is a single steady state $\bar{x} = 0$, and for $\mu > 0$, we obtain two steady states. Next, let us consider the local stability of the individual steady states. For $\mu = 0$, the system's equation is $\dot{x} = -x^2$. Thus, the steady state $\bar{x} = 0$ is unstable, with trajectories diverging away from the steady state for an initial condition $x_0 < 0$. However, for an initial condition $x_0 > 0$, trajectories in fact converge towards the steady state. In the case that $\mu > 0$, we can study stability of the steady states by considering the linear approximation of (5),

$$\dot{\xi} = -2\bar{x}\xi, \tag{7}$$

where $\xi = x - \bar{x}$ is the deviation from the steady state $\bar{x}$. Thus, the steady state $\bar{x}_1 = \sqrt{\mu}$ is exponentially stable, while the second steady state $\bar{x}_2 = -\sqrt{\mu}$ is unstable, with diverging trajectories in both directions. The dynamical behavior of the system (5) is illustrated with a bifurcation diagram in Figure 1.

In higher-dimensional systems, the saddle-node bifurcation takes place on a one-dimensional center manifold W^c. The dynamical behavior is illustrated in Figure 2. As in the one-dimensional case, there is no steady state for $\mu < 0$, and for $\mu = 0$ a steady state emerges, which then bifurcates into a stable and an unstable steady state for $\mu > 0$. Note that in the two-dimensional case, for $\mu > 0$, the stable steady state is a node, and the unstable steady state is a saddle. For $\mu = 0$, the saddle and the node merge and then disappear for $\mu < 0$, hence the name of the bifurcation.

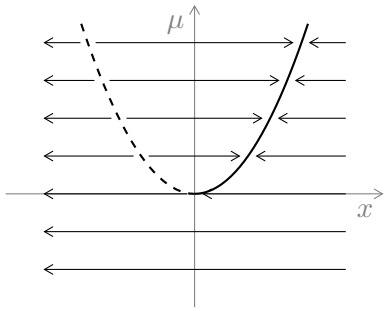

Fig. 1. Bifurcation diagram for the normal form of the scalar saddle node bifurcation (5).

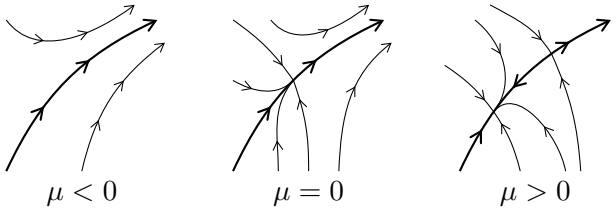

Fig. 2. Bifurcation diagram for the saddle-node bifurcation in a two-dimensional system. The bold line in the case $\mu = 0$ is the center manifold on which the bifurcation takes place.

B. Basic structure of genetic and biochemical switches

Next, let us consider how the saddle-node bifurcation is related to switching in biological systems. A key feature of biological switches is the occurence of at least two stable steady states in the system (1), which can be identified with two distinct operational modes of the corresponding biological process. Thus, biological switches are characterized by the property of bistability, i.e. the coexistence of two stable steady states [6]. In the generic case, where no specific biological function is assigned to the switch, we just refer to the two stable steady states as the on- and off-state of the switch. In fact, the usual structure of a biological switch is a system which may have three steady states: two distinct stable nodes, and one saddle lying "between" the stable steady states. More specifically, the saddle point is located on the separatrix, i.e. the boundary of the regions of attraction for the two stable steady states.

With the considered structure of the switch, the process of switching from off to on corresponds to the occurence of a saddle-node bifurcation of the off-state and the saddle point. Thus, the on-state remains as the only stable steady state, and the system will converge to this state. Conversely, switching from on to off corresponds to a saddle-node bifurcation of the on-state and the saddle point, and the system will converge to the off-state in this case. We next discuss how this mechanism is implemented in two common biochemical network motifs which appear in biological switches.

1) Switches with a single element: The simplest network structure giving rise to a biochemical or genetic switch is an autoregulatory element, i.e. a biochemical entity that reinforces its own activity. For a genetic switch, this entity is

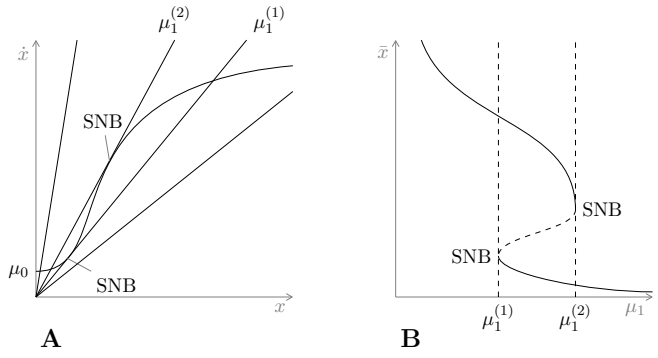

Fig. 3. Steady state illustration (A) and bifurcation diagram (B) for the scalar switch (9).

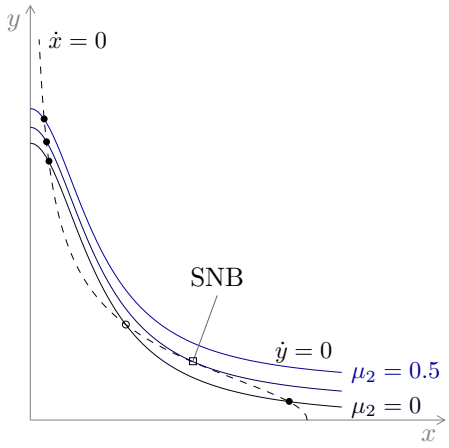

Fig. 4. Nullclines for the mutual repression switch for different values of μ_2. The saddle-node bifurcation is labelled with a square. Filled dots indicate stable steady states, the contoured dot an unstable steady state.

typically a transcription factor which positively regulates its own expression from the genome. In biochemical signalling networks, a typical motif is a kinase that catalyzes its own activation by phophorylation. In both cases, the motif can be modelled by the differential equation

$$\dot{X} = k_0 + \frac{V_m X^2}{K_m^2 + X^2} - k_d X, \tag{8}$$

where $X \in \mathbb{R}$ is the activity of the considered entity, and k_0, V_m, K_m, and k_d are positive parameters. Thereby, the first term k_0 corresponds to a basal production rate independent of the activity of X, the second term $\frac{V_m X^2}{K_m^2+X^2}$ to gene expression via transcription and translation in a genetic switch, and for example to phosphorylation in a biochemical signalling switch. The third term $k_d X$ corresponds to a linear decay, representing for example protein decay or dephosphorylation. The system is rescaled by setting $X = K_m x$, where x is the normalized activity of the considered entity, and introducing the normalized time $\tau = \frac{V_m}{K_m}$. The resulting normalized system is given by

$$\frac{dx}{d\tau} = \mu_0 + \frac{x^2}{1+x^2} - \mu_1 x, \tag{9}$$

where $\mu_0 = \frac{k_0}{V_m}$ and $\mu_1 = \frac{k_d K_m}{V_m}$ are dimensionless parameters.

The behavior of the system (9) in dependence of the parameters μ_0 and μ_1 can be illustrated by plotting the production terms and the degradation term over x, as in Figure 3A. From Figure 3A, it is clear that varying the parameter μ_1 yields two saddle-node bifurcations, one at $\mu_1^{(1)}$, the other at $\mu_1^{(2)}$. The dependence of the steady state on the parameter μ_1 is shown in the bifurcation diagram in Figure 3B. For $\mu_1 \leq \mu_1^{(1)}$, the only stable steady state is the one where $\bar{x}$ is high, thus the switch is said to be on. On the other hand, for $\mu_1 \geq \mu_1^{(2)}$, the only stable steady state is with low $\bar{x}$, and the switch is off in this case. For $\mu_1^{(1)} < \mu < \mu_1^{(2)}$, the system is bistable, and the state of the switch depends on whether μ_1 came into this interval from below or above.

2) Switches with two elements: Switches with two elements are commonly based either on mutual repression, i.e. each of the two elements represses the other's activity, or on mutual activation, i.e. the elements activate each other, similar to the autoregulatory motif discussed for the scalar switch (8). Here, we discuss only the mutual repression case. Let x and y denote the normalized concentrations or activities of two biochemical entities X and Y. The mutual repression is achieved by letting the production or activation of X decrease with increasing Y, and vice versa. Moreover, we assume that both entities are subject to a linear decay, and that for each component there is an additional production source independent of the other component's activity. A typical non-dimensionalized ODE model resulting from these model assumptions is given by

$$\begin{aligned} \dot{x} &= \frac{4}{1+y^2} - x + \mu_1 \\ \dot{y} &= \frac{4}{1+x^2} - y + \mu_2, \end{aligned} \tag{10}$$

where μ_1, $\mu_2 > 0$ are adjustable parameters representing the independent production source. In the case where $\mu_1 = \mu_2 = 0$, the system (10) is bistable, with the two stable steady states corresponding to either high x and low y or vice versa, as shown in Figure 4. The system can be forced into the state with high y by increasing the parameter μ_2: for a critical value of μ_2, the stable steady state with high x and the unstable steady state merge and disappear in a saddle-node bifurcation, as shown in Figure 4, and the system is forced to the state with high y. Conversely, the system can be forced to the state with high x by increasing μ_1. In this case, the state with high y and the unstable steady state would disappear via a saddle-node bifurcation.

C. Examples of genetic and biochemical switches

Genetic and biochemical switches based on bistability and the saddle-node bifurcation have emerged as a common motif that living cells use to regulate their internal state. The basic structures discussed in the previous section are indeed frequently encountered in intracellular networks. Yet, most actual switches are of larger complexity, owing to

the need for several regulatory inputs, increased robustness of bistability, or just the biochemical features required to generate the sigmoidal shape of the curves in Figures 3A and 4 [7]. Nevertheless, it is instructive to study a few typical examples of intracellular switches within the framework of the previous section.

A thoroughly studied example is the lac operon [8], which is a genetic switch used by intestinal bacteria in order to decide between the digestion of one of the two sugars lactose and glucose. The switch is based on the protein LacI, which represses transcription of the lac operon, encoding for lactose metabolism proteins. However, LacI is also inhibited by products of the lactose metabolism, yielding a switch based on mutual repression [9].

The process of programmed cell death, also called apoptosis, is an example where a biochemical switch is embedded within a larger network structure. Apoptosis is a mechanism present in all mammalian cells, which is essential to remove malfunctioning or unneeded cells from the organism in a coordinated manner. It can be triggered by extracellular or intracellular stimuli, and is essentially based on a mutual activation switch within the so called caspase cascade [10]. The switching behavior in apoptosis can be analyzed in a similar way as the switch with two elements discussed in Section III-B [7].

IV. THE HOPF BIFURCATION AND OSCILLATIONS

A. The Hopf bifurcation

Hopf bifurcations (HB) are the generic bifurcations for the generation of limit cycles in nonlinear systems. In a HB, a stable fixed point $\bar{x}$ is destabilized via a transition of a complex pair of eigenvalues of the Jacobian matrix $\mathcal{J}_f^\mu(\bar{x})$ from the left to the right half plane, which goes along with the emergence of a limit cycle. The normal form of a HB in cartesian coordinates is given by

$$\begin{pmatrix}\dot{x}_1\\ \dot{x}_2\end{pmatrix} = \begin{pmatrix}\mu & -\omega\\ \omega & \mu\end{pmatrix}\begin{pmatrix}x_1\\ x_2\end{pmatrix} + (x_1^2+x_2^2)a\begin{pmatrix}x_1\\ x_2\end{pmatrix} \qquad (11)$$

with parameter $\omega > 0$. It has a fixed point at $(\bar{x}_1, \bar{x}_2) = (0,0)$ with a complex conjugate pair of eigenvalues of the Jacobian matrix $\mathcal{J}_f^\mu(\bar{x})$ given by

$$\lambda_{1,2} = \mu \pm \sqrt{-\omega^2}. \qquad (12)$$

A HB occurs at $\mu^c = 0$, when the real parts of these eigenvalues change signs. It is convenient to transform equation (11) into polar coordinates,

$$r^2 = x_1^2 + x_2^2 \qquad x_1 = r\cos\theta \qquad (13)$$

$$\theta = \arctan\left(\tfrac{x_2}{x_1}\right) \qquad x_2 = r\sin\theta, \qquad (14)$$

which leads to

$$\dot{r} = \mu r + a r^3 \qquad (15)$$

$$\dot{\theta} = \omega. \qquad (16)$$

In this form, the radial and angular coordinates are decoupled and can be considered separately. Equation (16) describes a counterclockwise rotation with constant angular velocity ω. Considering $\dot{r}(a,\mu)$, we have to distinguish between two different cases:

1) Supercritical HB for $a < 0$ (Fig. 5 top): It is easy to verify that for $\mu < 0$, $\dot{r}$ has a single fixed point $\bar{r} = 0$, which is globally stable. Thus system (11) has a globally stable focus at the origin and exhibits damped oscillations. The focus becomes unstable at $\mu = 0$, and a globally stable limit cycle with radius $r_0 = \sqrt{\frac{-\mu}{a}}$ emerges. The system shows sustained sinusoidal oscillations (Figure 5 *top*). A general characteristic for supercritical HBs is the increase of the oscillation's amplitude and period near the HB with orders $\mathcal{O}(\mu^{1/2})$ and $\mathcal{O}(1)$, respectively.

2) Subcritical HB for $a > 0$ (Fig. 5 bottom): Similarly, $r_0 = 0$ is a fixed point of the system in any case. It is the only limit set if $\mu > 0$, and it is an unstable focus in this case. The system shows oscillations with increasing amplitude. The focus becomes stable for $\mu < 0$, and an unstable limit cycle with radius $r_0 = \sqrt{\frac{-\mu}{a}}$ emerges, which is the border of the basin of attraction of the stable focus. Depending on the initial condition, the system exhibits damped oscillations or, if $r(0) > r_0$, it oscillates with increasing amplitude (Figure 5 *bottom*). Since the r^3 term is destabilizing here, often higher order terms stabilize the solution in real systems. For example, adding a term r^5 to equation (15) creates a second limit cycle with large amplitude via a SNB of cycles at a value $\mu^c < 0$, which is stable.

Finally, we note that oscillations with constant angular velocity and decoupling of both polar coordinates, i.e. sinusoidal oscillations, are a specialty of the normal form, and generally things are more complex in this respect.

B. Biochemical oscillators in 2D

We start considering two-dimensional RNs, in which the occurence of limit cycles goes along with typical courses of nullclines. A negative circuit in the interaction graph with at least two nodes is necessary for oscillations [11], [12], since systems with graphs lacking negative circuits have a monotone flow with respect to a partial ordering induced by an orthant of the coordinate system (for more details see [13], [14], [15] and references therein). This property prevents in particular the existence of stable limit cycles and hence oscillations. A single negative circuit is however not sufficient to generate oscillations in two-dimensional systems: Since the sign of the real parts of the eigenvalues in $\mathcal{J}_f^\mu(x)$ are given by the sign of $tr(\mathcal{J}_f^\mu(x))$, which is always negative, the system cannot undergo a HB. One of the two components must activate its own state, which leads to a classification of two-dimensional biochemical oscillators into *activator-inhibitor oscillators* (AIO) and *substrate depletion oscillators* (SDO). These two groups are characterized by the sign pattern of the Jacobian matrix $J_f^\mu(x)$ near the fixed point $\bar{x}$, or, equivalently, the interaction graph topology, and the course of the nullclines in a neighborhood of $\bar{x}$. The sign

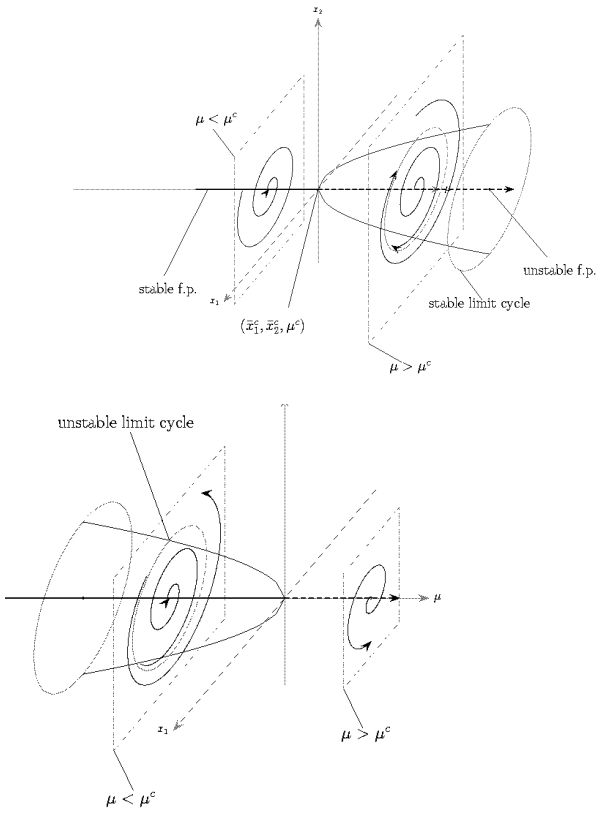

Fig. 5. Bifurcation diagrams for super- and subcritical Hopf bifurcations.

patterns are given by [16]:

$$\sigma^{AIO}\left(\mathcal{J}_f^\mu(x)\right) = \begin{pmatrix} + & - \\ + & - \end{pmatrix}, \sigma^{SDO}\left(\mathcal{J}_f^\mu(x)\right) = \begin{pmatrix} + & + \\ - & - \end{pmatrix}$$

for the AIO and the SDO, respectively. Self-activation is, similar to the SNB, usually described by a non-linear term that saturates for high concentration values, such that the linear degradation term dominates over the self-activation ($\frac{\partial \dot{x}}{\partial x} < 0$) for large concentrations in x. This stabilizes the system in the sense that forward trajectories are bounded and leads to typical courses of nullclines for AIOs and SDOs (Figures 6 and 7).

The existence of a stable limit cycle can be shown using the *Poincaré-Bendixson theorem* (PBT) for these systems [17], [18], [19]. This theorem states that if a trajectory $\Gamma(x)$ is confined to a closed and bounded region R that does not contain any fixed points of the system, $\Gamma(x)$ eventually approaches a closed orbit or is a closed orbit. In any case, R contains a closed orbit. In order to apply the PBT, we have to find bounds of such a region R. This is usually done in two steps: First, a trapping region, that is, a forward invariant closed and bounded set that is eventually reached by all forward trajectories, is constructed. The nullclines are often helpful here. Second, since both models contain a single fixed point inside the trapping region, this has to be eliminated by excluding a small disk with radius ϵ about $\bar{x}$. For R to be still positively invariant, all real parts of the eigenvalues of

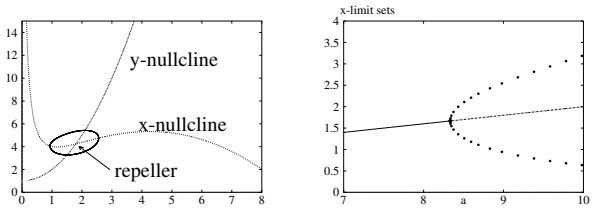

Fig. 6. *A.* Nullclines and limit cycle of the AIO (17) for parameter values $a = 9$ and $b = 2$. *B.* Bifurcation diagram with a supercritical HB.

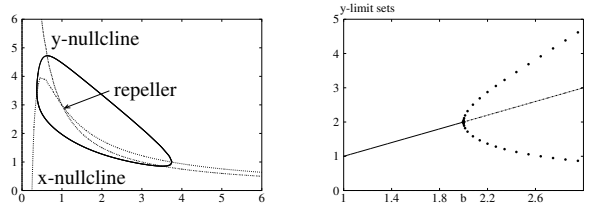

Fig. 7. *A.* Nullclines and limit cycle of the SDO (18) for parameter values $a = 1$ and $b = 3$. *B.* Bifurcation diagram with a supercritical HB.

$\mathcal{J}_f^\mu(\bar{x})$ have to be positive, which means that $\bar{x}$ must be a repeller. Thus, once a trapping region has been found, the existence of a closed orbit can be proven by studying the local phase portrait of the fixed point.

A representative model for an AIO is the *dioxide-iodine-malonic acid reaction*, which is similar to the well-known Belousov-Zhabotinsky reaction. A simplified non-dimensionalized version of this reaction reads [19]

$$\begin{aligned} \dot{x} &= a - x - \frac{4xy}{1+x^2}, \qquad a > 0 \\ \dot{y} &= bx\left(1 - \frac{y}{1+x^2}\right), \qquad b > 0, \end{aligned} \tag{17}$$

where x and y are the dimensionless concentrations of iodine and chlorine dioxid ions. The nullclines of system (17) are shown in Figure 6 for parameter values $a, b = (9, 2)$, along a bifurcation diagram with a as bifurcation parameter. The system undergoes a supercritical HB.

The *brusselator* is a well-known SDO model with vector fields given by polynomials,

$$\begin{aligned} \dot{x} &= a + x^2 y - (b+1)x \\ \dot{y} &= bx - x^2 y. \end{aligned} \tag{18}$$

Courses of nullclines and the stable limit cycle for parameter values $a, b = (1, 3)$ and the bifurcation diagram over the parameter b are shown in Figure 7.

It should be mentioned that the course of the nullclines of AIO and SDO models are typical for many two-dimensional models that show sustained oscillations such as predator-prey systems like the Lotka-Volterra model (see for example Chapter 3 in [20]), the FitzHugh-Nagumo model from the Hodgkin-Huxley theory of nerve membranes (more details in Chapter 7 of [20]), or biochemical network models such as described in [21], [22]. Furthermore, the PBT has been generalized to higher dimensions for networks with certain topology [23], [24], [25]. There are, however, only few

results about the existence of limit cycles for arbitrary network topologies.

C. Robustness of biological oscillators

Cellular rhythms, i.e. periodic behavior caused by interactions between molecules, play important roles for living systems. Examples for such intracellular oscillations that have extensively been investigated both theoretically and experimentally are circadian rhythms [26], [22], calcium oscillations (e.g. [27], [28], [20]) or the cell cycle [29], [30], [31]. For an introduction into biological oscillator models we refer to [32], [33], [16]. The onset of theoretical models for oscillating gene-protein networks was provided by Goodwin [34], who proposed a minimal model of three variables, a gene, protein and end product that inhibits its own transcription. Thus the three variables are connected in a single negative feedback loop. In contrast to two-dimensional models, in three and more dimensions a single negative circuit in the interaction graph can already give rise to Hopf bifurcations and hence show periodic behavior.

Research that combines theoretical investigations on these minimal models and experimental observations has created design principles for synthetic biological oscillators in living systems. Among the first of these was the repressilator that consists of three mutually inhibiting gene products in *Escherichia coli* [35]. A more complex synthetic gene-metabolic oscillator that causes periodic behavior of a huge amount of metabolites in *E. coli* was designed by [36].

A comparison with the minimal theoretical models just described and experimental observations has revealed several phenomena that cannot be captured well by those models. Robustness of oscillations with respect to perturbations in initial conditions and parameter variations, or the ability to maintain a stable period and amplitude under fluctuating conditions, are important issues in this context (see for example [26], [37], [22], [38], [39]). For two- or three variable models, stable limit cycles only exist in a small region of the parameter space that is bounded by Hopf bifurcations [32]. The conditions for a Hopf bifurcation to occur are especially restrictive for the AIO and SDO oscillator models, for example, the fixed point must lie between the maximum and minimum of the first nullcline [40], [41].

Different mechanisms are known that can stabilize periodic behavior. The most often investigated among these are time-delays (e.g. [21], [5], [22], [39], [42]) and large time-scale differences (e.g. [21], [40], [41], [25]). The latter are particularly relevant for biochemical networks that naturally include processes at different time scales. Transcription and translation are for example on a scale of minutes and hours and slow compared to posttranscriptional modifications of proteins or protein-DNA interactions, which are assumed to be in equilibrium after seconds [1]. For AIO and SDO models that have a stable fixed point, multiplication of the y-vector field with a sufficiently small time scale parameter ϵ creates a limit cycle by a Hopf bifurcation [40]. These are known as *relaxation oscillations* and characterized by two time scales: The system moves slowly along the slow manifold given by the x-nullcline, where x_1 is in a quasi-steady state, and makes fast transitions at the local minimum and maximum of this manifold during which only the fast component x_1 changes considerably.

Introducing a time-delay τ into the system makes analysis generally much more complex and requires the use of techniques for delay-differential equations. Fixed points $\bar{x}$ are however independent of the delay, and their stability can also be analyzed via linearization. The only difference to ordinary differential equations is that the characteristic equation is not a polynomial in the eigenvalues λ any more but a transcendental equation with an infinite spectrum of eigenvalues. Introducing time-delays is more efficient in stabilizing oscillations than large time-scale differences, and some of the conditions that are necessary for the occurence of Hopf bifurcations in the ODE model can be overcome by time-delays. Periodic behavior is for example possible in a one-variable model with delayed negative feedback or a two-variable model with a single negative feedback circuit. Moreover, the strong necessary condition for the fixed point of AIO and SDO models to lie between the local extrema of the x-nullcline is not required any more.

Besides increasing the parameter region in which the system has a stable limit cycle, both large time-scale differences and time-delays usually also increase amplitude and period of the oscillations.

There is evidence that more complex networks with interrelated feedback circuits and coupling of oscillators can also make oscillations more robustness to perturbations [37], [43], [38], [39]. Most of these studies are heuristic, which raises the question of about what can be generalized to arbitrary network structures and kinetics.

V. CONCLUSIONS

With this contribution we intended to give a tutorial to understanding the emergence of complex dynamic behavior in intracellular networks, with a particular focus on biological switches and oscillations. These are generically caused by saddle-node and Hopf bifurcations. We started with an introduction about the normal forms for those bifurcations, and then discussed their role in the context of biological network examples.

A prerequisite for the emergence of bifurcations in biological networks are the existence of nonlinear feedback, and minimal models for switches or sustained oscillations consist of positive auto-regulation and negative feedback between two components. While many subsystems that include such feedback structures have been identified to be responsible for complex behavior, several studies indicate that those core models alone are less robust than the real biological systems. Moreover, they often do not capture all features of the real systems like robustness of amplitude and period of oscillations. Consequently, it seems that more complex network structures with interrelated feedback circuits contribute to the robustness and stability of biological systems, and analysis methods for studying robustness in these larger networks is an important topic of current research in this field.

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