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Classical mechanics approach applied to analysis of genetic oscillators

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Abstract—Biological oscillators present a fundamental part of several regulatory mechanisms that control the response of various biological systems. Several analytical approaches for their analysis have been reported recently. They are, however, limited to only specific oscillator topologies and/or to giving only qualitative answers, i.e., is the dynamics of an oscillator given the parameter space oscillatory or not. Here we present a general analytical approach that can be applied to the analysis of biological oscillators. It relies on the projection of biological systems to classical mechanics systems. The approach is able to provide us with relatively accurate results in the meaning of type of behaviour system reflects (i.e. oscillatory or not) and periods of potential oscillations without the necessity to conduct expensive numerical simulations. We demonstrate and verify the proposed approach on three different implementations of amplified negative feedback oscillator.

Index Terms—Oscillatory Dynamics, Genetic Oscillators, Ordinary Differential Equations, Dynamical Systems.

1 Introduction

Analysis of biological oscillators is important in context of natural as well as synthetic biological systems. *In-silico* investigation of behaviour of different synthetic topologies that reflect oscillatory behaviour in certain conditions is able to guide the implementation of biological systems with desired functionality [1], [2]. Analysis of natural oscillators is, on the other hand, important for understanding of underlying mechanisms that regulate several cellular responses, such as circadian clocks or cell cycle oscillators [3], [4].

Genetic oscillator analyses have been a subject of in-depth investigation recently following the first successful *in vivo* implementation of the repressilator circuit [5]. The repressilator and its generalized models were thoroughly analysed by different authors [6], [7], [8]. Comparative analysis of design principles in biochemical oscillators, oscillator topologies or only variations in their implementations have also been performed by several researchers [1], [2], [9], [10]. The most straightforward approach to the quantitative analysis of oscillatory behaviour used in existent studies is to perform numerical simulations on different oscillator models, and analyse the time evolution of observed chemical species in dependence on chemical kinetic rates and initial conditions (for example see [10]). However, main disadvantages of this approach are in inherent expensiveness of numerical simulations and additionally in dependence on initial conditions, i.e. on initial species concentrations.

Another strategy is to use various analytical approaches which do not rely on repetitive iterations of expensive numerical simulations. These are in most cases limited to only specific systems and/or bifurcation types. For example, search for the existence of stable limit cycles can be performed analytically. It is very difficult to determine whether the equations that describe the system will reflect oscillatory behaviour or not in general [11]. In some cases, however, it is possible to determine parameter ranges for which the system exhibits oscillatory behaviour. Repressilator topology, for example, only exhibits Hopf bifurcation and can be analysed thoroughly with the linearisation of its model and investigation of the eigenvalues of the linearised system [5], [6]. It is also possible to evaluate oscillation periods near the bifurcation points when oscillations emerge from a supercritical Hopf bifurcation. If other types of bifurcations occur, or if we are interested in the dynamics far from bifurcation points, other methods need to be applied.

Existing analytical approaches mostly allow us to only perform the qualitative analysis, e.g., see [12]. Some efforts have been made recently to also obtain the quantitative information of oscillatory response. Analytical approximation of oscillation frequency and period derived specifically for two different oscillator topologies, i.e. circadian oscillator model according to [13] and repressilator model according to [5], has been reported [14]. Authors show that the oscillation frequency and period can be approximated with a satisfactory accuracy with analytical investigation of ordinary differential equations describing the system's dynamics. Approach presented is, however, limited to only two different topologies. In [15] authors present an analytical method that can be applied to a genetic oscillator with negative feedback ring topology, i.e. a more generalized model which can also describe the Goodwin oscillator and repressilator [5], [16]. This again presents a limitation because the method cannot be straightforwardly applied to more general topologies which also include positive feedbacks. Positive feedback loops, however, increase the chances of obtaining oscilla-

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tions [17], [18], [19], allow the tunability of the oscillation periods [20], and also introduce a certain degree of nonlinearity in the system [21]. Moreover, approaches described in [14] and [15] rely on the approximation of a promoter activity with a Boolean variable having two possible values, i.e. zero (not active) and one (active). Promoter dynamics is thus approximated with a unit step function, which yields active promoter when activator threshold concentration value is exceeded by activators, and/or repressor threshold concentration value is not exceeded by repressors at the same time. This presumption is valid only for a very limited scope of biological systems, i.e. for promoters that exhibit highly nonlinear response, and is also unable to differ among certain promoter implementations, e.g., competitive versus non-competitive transcription factor binding (see Supplementary material).

Here we present a more general analytical approach, which can be applied to an arbitrary oscillator topology. It relies on the projection of the model describing the dynamics of a biological system to a classical mechanics system. The projected system can be analysed further on with classical mechanics theory. Described approach is able to provide us with the type of behaviour system reflects (oscillatory or stationary) and periods of potential oscillation without the necessity to conduct expensive numerical simulations. This allows us to perform the analysis more efficiently and with less computational effort than with conventional modelling and analysis methodologies. The advantages of proposed methodology become evident especially when we are interested in the dynamics of the system in dependence on a vast range of parameter values, e.g., when conducting local or global sensitivity analysis [22], parameter sweep analysis, or when exact parameter values are unknown. In the latter case, e.g., we need to investigate the dynamics of the system using a large scope of different parameter values surrounding presumed nominal parameter estimates. The methodology can be as such efficiently applied to the process of design of novel genetic oscillators and also to the understanding of existing ones, e.g., in the context of their sensitivity to environmental changes. Even though we focus our attention to genetic oscillators only, the proposed methodology can also be used to analyse other types of biological or chemical oscillators or even dynamical systems in general.

The rest of the paper is organized in the following way. First we introduce and describe the proposed application of classical mechanics to biological systems in details. We demonstrate the methodology on three different amplified negative feedback oscillator models derived from [10]. We verify the proposed approach with the comparison performed on the results obtained with numerical simulations. We conclude the paper with the discussion about the advantages and drawbacks, and give some concrete biological applications and possible future improvements of proposed approach.

2 METHODS

2.1 Biological systems as dynamical systems

One of the most common approaches for modelling the dynamics of biological systems, especially gene regulatory networks, is deterministic modelling. In this case models are established on the basis of a system of coupled ordinary differential equations (ODEs) [23], [24], which approximate an average response of the network deterministically [25]. These equations describe the dynamics of each observed chemical species in the following way:

$$\frac{dx_i}{dt} = f_i(\mathbf{x}(t), \mathbf{p}), \tag{1}$$

where f_i is a (usually non-linear) function governing the dynamics of chemical species x_i ($i \in \{1, 2, ..., n\}$) in dependence on the state of the system in time step t, i.e. $\mathbf{x}(t) = (x_1(t), x_2(t), ..., x_n(t))$ and in dependence on model parameters $\mathbf{p} = (p_1, p_2, ...)$. These equations can be established with the basic mass action kinetics, and can be further simplified using, e.g., Michaelis-Menten equations to approximate enzymatic reactions or Hill equations to describe gene expression [26]. These can be additionally generalized with fractional occupancy [27] or thermodynamic modelling approach [28]. Due to the nonlinearity of ODEs, their solutions are usually obtained with numerical integration. The main problems of this approach are in dependence on initial conditions and in its computational expensiveness. Computational expensiveness presents a major obstacle especially when parameter values are only partially known, or when we are interested in the stability of system's reponse in dependence on parameter perturbations. In these two cases large amount of simulations needs to be performed in order to obtain response of the system to a vast range of different parameter values. Analytical approaches that approximate the phenotype behind the ODE description of the system without the necessity to conduct expensive numerical simulations have already been proposed for certain biological system (e.g., see [14], [15] for selected genetic oscillators). State-of-the-art approaches, however, lack generality while they can be applied straightforwardly to only selected network topologies, and also make certain presumptions that additionally limit the scope of their usability (e.g., using unit step function to approximate promoter activity - see Supplementary material). We introduce a more general approach that can be applied to an arbitrary biological oscillator topology to analytically determine its qualitative and quantitative response.

2.2 Oscillatory behaviour in classical mechanics

Classical mechanics defines oscillatory behaviour as periodic changes in body position within the observed space. It occurs in the neighbourhood of some stable position, i.e. *equilibrium point* (EP), in which either no forces act on the body, or all the forces are compensated. Oscillatory behaviour occurs if small perturbation from EP causes the occurrence of the force in the direction of EP, i.e. returning force [29]. Let's consider a 1-dimensional mechanical system along unique coordinate axis x. In this case all vector quantities (such as force) can be used in terms of their projection. If direction of vector coincides with direction of axes, this vector has a positive projection, otherwise a negative one.

Equations describing the motion are usually given in specific forms. Solutions of these equations can be interpreted as the positions of body in motion in dependence on time. This motion is caused by the forces that result in

the body acceleration, and can be expressed as the second time derivative of the body position:

$$a = \ddot{x}. \tag{2}$$

Equations describing the motion in classical mechanics are therefore usually given in the form of the second order differential equations. Since force F is a function of coordinate x in a 1-dimensional system, EPs (i.e. x_0) can be determined with the solutions of the equation $F(x_0) = 0$. We can conduct the analysis of the system in the neighbourhood of the EP with the Taylor series expansion:

$$F(x) \approx F(x_0) + (x - x_0)\frac{dF}{dx}\bigg|_{x=x_0} + ... \tag{3}$$

Zero-order term equals zero as long as x_0 is an EP. Higher-order terms can be neglected in the close proximity of the EP. The dynamics in the neighbourhood of the EP is therefore defined by the first order terms only. Their positive values indicate the force directed from the EP. On the contrary, their negative values indicate the existence of the returning force that presents a necessary condition for the oscillatory behaviour. Let's define k as an absolute value of first-order derivative of F in EP:

$$k = \left| \frac{dF}{dx}\bigg|_{x=x_0} \right|, \tag{4}$$

which equals

$$k = -\frac{dF}{dx}\bigg|_{x=x_0} \tag{5}$$

in the case of the returning force around the EP. The substitution of Eq. (5) and equation $F = ma$, where m presents the body mass, to Eqs. (2) and (3) yields the following equation

$$m\ddot{x} = -k(x - x_0), \tag{6}$$

which can be rewritten as

$$\ddot{x} = -\omega^2 x. \tag{7}$$

Here $\omega^2 = \frac{k}{m}$. It is important to note that ω can be interpreted as the circular frequency of oscillations. The equation can be used to express the period of oscillations

$$T = \frac{2\pi}{\omega} = 2\pi\sqrt{\frac{m}{k}} \tag{8}$$

as a well-known formula for period of spring pendulum, where k can be interpreted as an elastic coefficient of the spring.

Another approach to determine the motion is to use potential energy function, i.e. a function of coordinates ($U = U(x, y)$ in 2D-case) linked with the forces acting on the system by the following relations:

$$\begin{aligned} F_x = m\ddot{x} = -\frac{\partial U}{\partial x}, \\ F_y = m\ddot{y} = -\frac{\partial U}{\partial y}. \end{aligned} \tag{9}$$

Potential energy can be visualized as a surface with some relief, on which the system can move. Minimums of the relief present the points of stable equilibrium

$$\begin{aligned} U(x, y) - \ \min \text{ in } x_0, y_0 \equiv \\ U'_x = 0 \text{ and } U'_y = 0 \equiv \\ F_x = F_y = 0. \end{aligned} \tag{10}$$

The potential energy has the following form

$$U(x, y) = \frac{a(x - x_0)^2}{2} + \frac{b(y - y_0)^2}{2}. \tag{11}$$

for a simple oscillator. Partial derivation of this formula leads to expression of forces in the form of Equation (6). In this expression a and b can be interpreted as the squares of circular frequencies ω_x^2 and ω_y^2 (presuming that m equals 1), from which period can be calculated by Equation (8). Naturally, a and b are positive parameters.

System with such form of potential energy function is called a *harmonic oscillator*. It presents the simplest system which may exhibit oscillatory behaviour, and may be used to approximate different oscillators. We also use such idealized model as an approximation of our original system. The approximation, however, cannot be performed when no EPs exist. In that case we can immediately deduce that the behaviour of the system is not oscillatory (cases with negative EPs can also be eliminated in the case of biological systems while the concentrations should always be non-negative). Further on, we observe the potential energy of an approximated system, which presents a necessary condition for oscillations to occur. In case the energy is not conserved or when potential energy function cannot be expressed, the system will exhibit damped or no oscillation at all (see Sect. 2.3 for further details).

2.3 Classical mechanics in dynamical systems

Dynamical systems can be in general described with the system of first order differential equations (ODEs), which include variables that may have different interpretation. In physics, these equations describe mechanical motion, where variables are interpreted as coordinates of body position. In biological systems, these variables usually describe the dynamics of observed chemical species (see Sect. 2.1). Our methodology relies on the approximation of system's potential energy function, which can be performed for an arbitrary oscillatory dynamical system with the calculation of first integral. It therefore does not depend on the background behind the ODE system. Here, however, we focus to the analysis of biological oscillators.

Let's presume we are observing the biological system which is comprised of only two chemical species. The dynamics of this system can be described with the following set of equations

$$\begin{aligned} \dot{x} = f(x, y, \mathbf{p}) = f, \\ \dot{y} = g(x, y, \mathbf{p}) = g. \end{aligned} \tag{12}$$

The time derivation of these equations brings them to the form of the Equation (7)

$$\begin{aligned} \ddot{x} = f'_x\dot{x} + f'_y\dot{y}, \\ \ddot{y} = g'_x\dot{x} + g'_y\dot{y}. \end{aligned} \tag{13}$$

According to Leibniz rule, we obtain

$$\begin{aligned} \ddot{x} = f'_x f + f'_y g \equiv \tilde{f}(x, y), \\ \ddot{y} = g'_x f + g'_y g \equiv \tilde{g}(x, y). \end{aligned} \tag{14}$$

These equations can be expanded in the Taylor series. Only first order terms need to be regarded here (zero order terms equal 0 in the EPs and higher order terms can be neglected):

$$\begin{aligned}\ddot{x} &= \frac{\partial \tilde{f}}{\partial x}|_{x_0,y_0}(x-x_0)+\frac{\partial \tilde{f}}{\partial y}|_{x_0,y_0}(y-y_0)\\ &\equiv a(x-x_0)+b(y-y_0),\\ \ddot{y} &= \frac{\partial \tilde{g}}{\partial x}|_{x_0,y_0}(x-x_0)+\frac{\partial \tilde{g}}{\partial y}|_{x_0,y_0}(y-y_0)\\ &\equiv c(x-x_0)+d(y-y_0).\end{aligned} \tag{15}$$

Potential energy for such equations system exists if $b = c$ because

$$\frac{\partial^2 U}{\partial x \partial y} = \frac{\partial^2 U}{\partial y \partial x}. \tag{16}$$

If this condition is not satisfied, the potential energy function does not exist, and the behaviour of the simplified system cannot be oscillatory. Otherwise the potential energy has the following form

$$U(x,y) = -\frac{a(x-x_0)^2}{2} - b(x-x_0)(y-y_0) - \frac{d(y-y_0)^2}{2}. \tag{17}$$

As bilinear form, this function can be diagonalized by rotation of coordinate system. This operation is performed by the calculation of eigenvalues of bilinear form matrix:

$$U(u,v) = \frac{\lambda_1(u-u_0)^2}{2} + \frac{\lambda_2(v-v_0)^2}{2}, \tag{18}$$

where u and v present the new coordinates after rotation, and $\lambda_{1,2}$ present the eigenvalues of potential energy matrix, which can be interpreted as the squares of circular frequencies of oscillations modes along new axes.

It is possible for the calculated circular frequencies to have non-zero imaginary parts, which consequences in the absorption of energy and damped oscillations. However, oscillatory behaviour can be achieved also with non-zero imaginary parts, which should be substantially smaller than real parts. Maximal threshold determining the ratio between imaginary and real parts of circular frequencies, for which oscillatory behaviour is still observed needs to be evaluated. Threshold value that we use in the analysis should minimize the errors of our approach in comparison to a credible source of data, e.g., numerical simulations, on a testing set of parameter values. The process of its estimation for the purpose of our case study is described in the Supplementary material. The same value can be used on all three models or even in a general case. One can alternatively determine specific threshold values that are optimal for a given model to obtain more accurate results. Real parts of obtained circular frequencies should be positive numbers at the same time, due to their biological interpretation.

Obtained values of circular frequencies can thus be used to determine the type of behaviour the system reflects (oscillatory or not), and to determine the periods of potential oscillations. Oscillation periods can be estimated in the following way. Two oscillation modes exist in a 2-dimensional system. Their periods can be expressed as

$$T_{1,2} = \frac{2\pi}{\sqrt{\lambda_{1,2}}}. \tag{19}$$

It is possible to obtain different values of periods for each oscillation mode (i.e., in the case when real parts of obtained eigenvalues are different). These can be used to calculate a single true value of oscillation periods which is experimentally observed, and which presents a minimal time interval needed for the system to periodically return to a state that was already reached before. Composition of oscillations along two orthogonal axes can be visualized, and this visualization is known as *Lissajous curves* [30]. Let's presume that oscillations happen with equal frequencies, i.e. both oscillation modes (both dimensions) have the same oscillation periods, and that the point is in equilibrium state along one axis (x) and maximally deflected along another one (y) at the same time. In quarter of period, coordinate x reaches the maximal amplitude value, and coordinate y returns to zero. Coordinate y continues decreasing up to maximal negative deflection. At the same time, coordinate x changes back to zero. Observed point therefore draws a circle on the coordinate plane. In general the Lissajous curves are closed curves if frequency ratios are rational numbers. Mathematically this means that some finite time interval exists in which the state vector periodically returns back to a state that was already reached before. This time interval presents the oscillation period of the system. Periods of oscillation, either observed on a point of mass or on a set of biological chemical species, can be therefore calculated as a Least Common Multiplier (LCM) of periods along separate axis defined in the following way:

$$T = LCM(T_1,T_2) | T,T_1,T_2 \in \mathbb{R}, \text{ if } \frac{T}{T_1}, \frac{T}{T_2} \in \mathbb{N}. \tag{20}$$

Here, oscillation periods of the system and oscillation periods of each of its dimensions are in general non-integer numbers. However, the definition requires that the ratios among them are integers.LCM of two rational numbers can also be obtained in the following, more convenient, way:

$$LCM(T_1,T_2) = \frac{LCM(m_1,m_2)}{GCF(n_1,n_2)}, \tag{21}$$

where $T_1 = m_1/n_1$ and $T_2 = m_2/n_2$ and GCF presents the greatest common factor of its arguments (see Supplementary material for further details about the calculation of the LCM of two non-integer numbers). This brings us to the last step of proposed methodology. In order to find true, observed periods of oscillation, LCM of periods along separate axes (i.e. periods of different modes) found in previous steps should be calculated.

Even though we described the proposed methodology on a two-dimensional system only, it can be applied to a system with more dimensions in the same way. In this case the number of oscillation modes equals the number of equations describing the system's dynamics. The oscillation periods are thus expressed with the LCM of periods of all modes.

3 RESULTS

3.1 Case study

The case study will be performed on an amplified negative feedback oscillator, which presents a very general topology including both positive and negative feedbacks. While this

topology has already been studied extensively, some of its variations have also been constructed experimentally (for example see [17] and [31]). Our analysis will be performed on selected implementations described in [10], namely two variations of Design I (see Fig. 1(a) and Fig. 1(b)) and one variation of Design III (see Fig. 1(c)). We only give a brief description of the models in the main text. More accurate derivation of these models accompanied with the nominal parameter values is included in Supplementary material of the manuscript (see also references [2] and [10]).

Design I implementation presumes that regulation of the activator protein is achieved through the competition between the activator and repressor at the activator's promoter. The implementation can be described with the model

$$\begin{aligned} \frac{dx}{d\tau} &= \Delta\left(\beta\frac{1+\alpha x^n}{1+x^n+\sigma y^m}-x\right), \\ \frac{dy}{d\tau} &= \Delta\gamma\frac{1+\alpha x^n}{1+x^n}-y, \end{aligned} \tag{22}$$

where x and y are non-dimensional variables associated to the activator and repressor, α presents the transcriptional synergy of activated promoter, β and γ present the non-dimensional strength of the transcription and translation for the activator and repressor, σ presents the binding ratio of repressor compared to activator, n and m present the Hill coefficients of activator and repressor, Δ presents the ratio between the activator's and repressor's degradation rates, and τ presents a non-dimensional time variable (see Supplementary material).

A variation of Design I (Design I.1) presumes additional positive feedback with the autocatalysis on the repressor. This implementation is also known as Smolen oscillator [1], [32]. It can be described with the model

$$\begin{aligned} \frac{dx}{d\tau} &= \Delta\left(\beta\frac{1+\alpha x^n}{1+x^n+\sigma y^m}-x\right), \\ \frac{dy}{d\tau} &= \Delta\gamma\frac{1+\alpha x^n}{1+x^n+\sigma y^m}-y. \end{aligned} \tag{23}$$

Here, the parameters have the same interpretation as in Design I.

Design III presumes specific inhibition, where activator and repressor bind to their designated binding sites, i.e. both binding sites at the activator's promoter can be occupied at the same time. It can be described with the model

$$\begin{aligned} \frac{dx}{d\tau} &= \Delta\left(\beta\frac{1+\alpha x^n}{(1+x^n)(1+\sigma y^m)}-x\right), \\ \frac{dy}{d\tau} &= \Delta\gamma\frac{1+\alpha x^n}{1+x^n}-y, \end{aligned} \tag{24}$$

where the parameters have the same interpretation as in Design I.

3.2 Analysis description

The analysis using the proposed methodology is performed in two steps. In the first step we determine the coordinates of EPs from the numerical solutions of

$$\frac{dx}{d\tau}=0,\ \frac{dy}{d\tau}=0. \tag{25}$$

In the second step we construct a potential energy matrix

$$\begin{bmatrix} -a & -b \\ -c & -d \end{bmatrix},$$

which is obtained with the derivations of initial ODE system $\frac{dx}{d\tau}$ and $\frac{dy}{d\tau}$ (see Equation (15)). First we examine the symmetry of the potential energy matrix (i.e. we check the condition $b = c$). If the condition is not satisfied, the behaviour of the system is identified to be stationary. Otherwise we further calculate the eigenvalues of potential energy matrix (λ_1 and λ_2), which present the squares of circular frequencies of two oscillation modes. Oscillatory behaviour is obtained only if the complex parts of these values are substantially small (see Sect. Evaluating the threshold values in Supplementary material). In this case periods of both oscillation modes can be expressed with the equation $T_{1,2} = 2\pi/Re(\lambda_{1,2})$. Finally, oscillation period is calculated as the LCM of periods of both modes.

Numerical analysis was performed additionally to verify the results of proposed methodology. It was conducted with the numerical integration of ODEs described in Sect. 3.1. The oscillatory behaviour was analysed on the time evolution of observed proteins' concentrations. In order to disregard the initial transient effects the signal was analysed from the second half of the simulation time onwards. Simulation time was sufficiently long for the transient effects to die out in each analysed scenario. The analysis was performed with the localization of the signal peaks and comparison of time delays between the peaks. If time delays were regular enough, the dynamics of the signal was treated as periodic.

Most of the calculations were performed with MATLAB. Numerical integration and the analysis of the results obtained with the integration were performed with GNU C Compiler (gcc) and GNU Scientific Library (GSL) [33]. Numerical analysis was based on the improved code provided in the Supplementary material of [10]. All the code used in this paper is available at http://lrss.fri.uni-lj.si/bio/material/oscmech.zip under the Creative Commons Attribution license.

3.3 Results

The analysis was performed on above described implementations of amplified negative feedback oscillator in dependence on parameters β and γ as shown in Figs. 2-4. Here, the periods of oscillations are presented in logarithmic scales. In each figure sub-figure (a) corresponds to classical mechanics method and sub-figure (b) to results obtained with numerical simulations.

Dependence of periods on parameters β and γ for Design I is presented in Fig. 2. Values of other parameters are $\alpha = 50$, $\Delta = 10$, $\sigma = 1$, $n = 2$ and $m = 2$. In Design I.1 oscillations occur for extended range of parameters β and γ. The results of the analysis are presented in Fig. 3). Values of other parameters are $\alpha = 50$ and $\Delta = 4$, $\sigma = 1$, $n = 2$ and $m = 2$. The model of Design III also exhibits oscillatory behaviour for a larger range of parameters β and γ than Design I as can be seen in Fig. 4. Values of other parameters are $\alpha = 50$, $\Delta = 1$, $\sigma = 1$, $n = 3$ and $m = 2$.

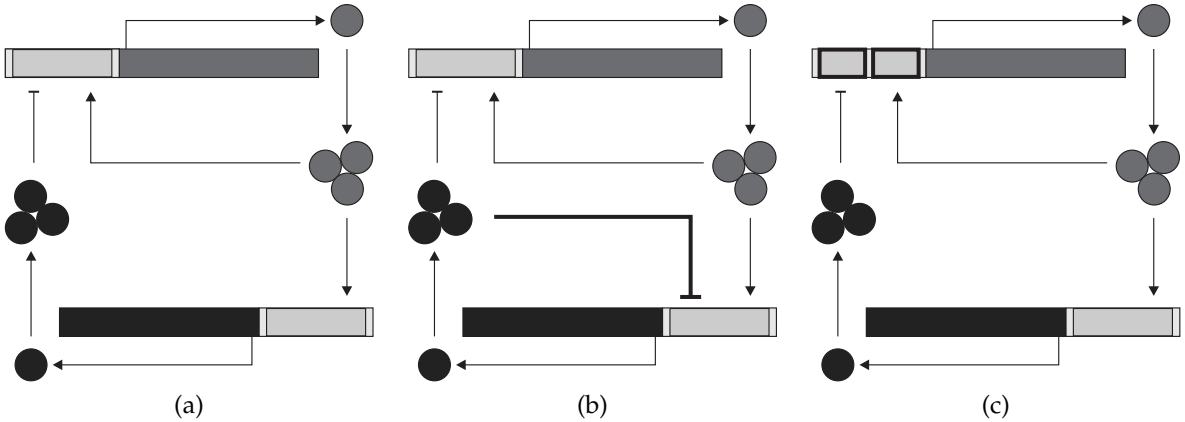


Fig. 1. Three different implementations of the amplified negative feedback oscillator that will be analysed, i.e. implementation with competitive binding of repressor and activator at the activator promoter, namely Design I (a), a variation of Design I with competitive binding of repressor and activator at the repressor promoter also, namely Design I.1 (b) and implementation without competition on either of promoters, namely Design III (c). Differences between the designs are emphasized.

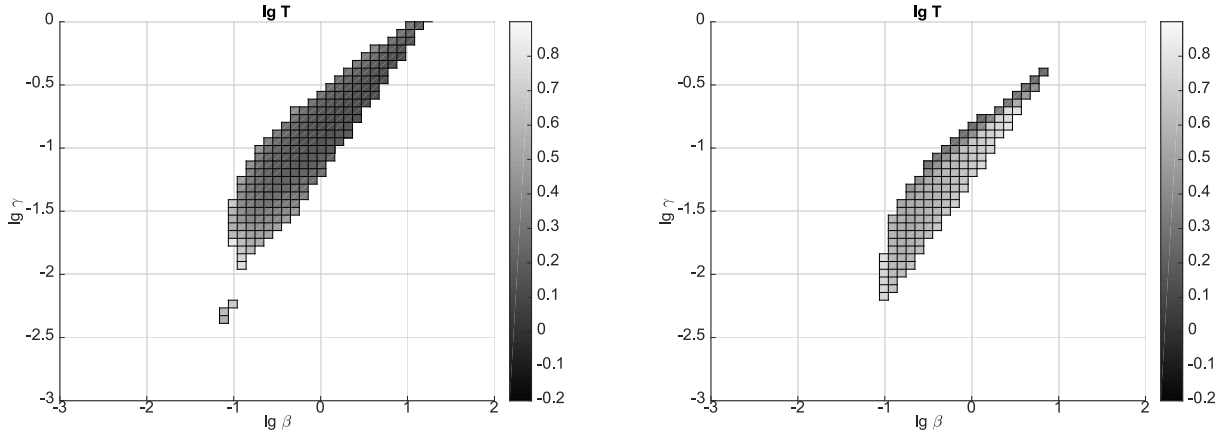


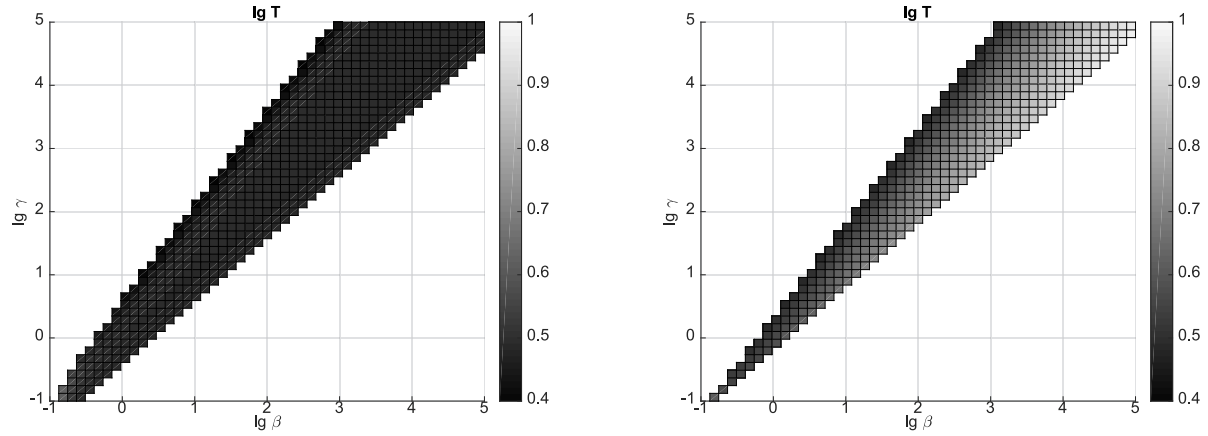
Fig. 2. Periods of oscillations in the model of Design I according to classical mechanics method (a) and numerical method (b), where $\alpha = 50$, $\Delta = 10$, $\sigma = 1$, $n = 2$ and $m = 2$. White colour denotes stationary behaviour.

4 Conclusion

Existing analytical methods are in general cases capable of answering the qualitative questions only, i.e., given the parameter values is the behaviour of the system under study oscillatory or not. Recently, several analytical methods for quantitative analysis have been developed which can be, however, applied only to limited oscillator topologies, and have a very limited biological relevance due to the rough approximations they make in the process of model simplification. We introduced a general analytical method that is capable to respond with the qualitative and quantitative answer with an estimation of oscillation periods. We described a classical mechanics approach that can be used for the analysis of the oscillatory behaviour in biological oscillators modelled with the system of ordinary differential equations. Proposed approach is based on the transformation of an oscillator's model to a classical mechanics system, more precisely to a harmonic oscillator. The methodology was established to analyse the transformed system from the qualitative, i.e., is the behaviour oscillatory or static, as well as from the quantitative perspective, i.e., what are the periods of observed oscillations. The approach was verified on three different implementations of the amplified negative feedback oscillator.

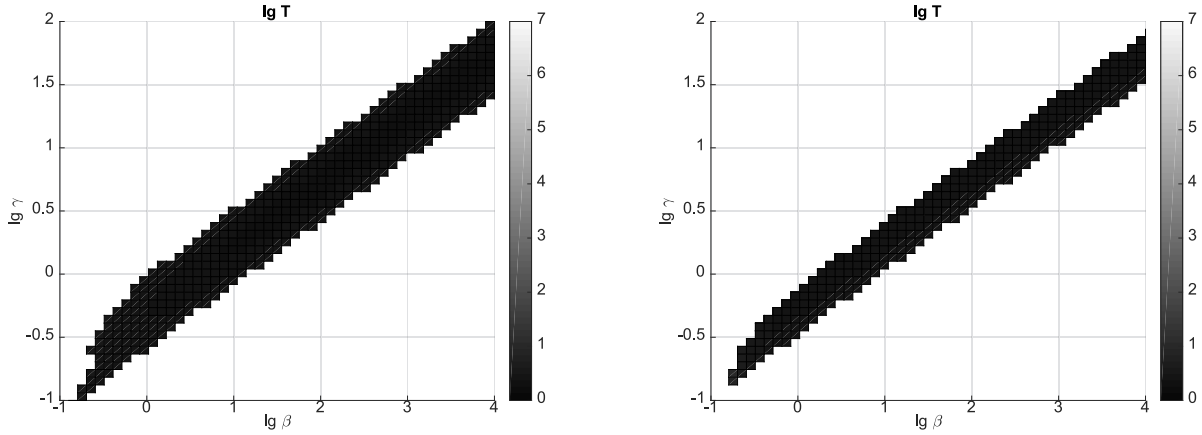
Proposed method is numerically stable while all mathematical operations performed in the analysis transform continuous functions to continuous functions (except derivation, which transforms smooth functions to continuous functions). This means that there is not any cause for divergence or instabilities related to parameters fluctuations if initial ODEs are smooth enough, which is always the case in the models in our focus.

Even though relatively accurate approximations of oscillatory behaviour were obtained with the proposed methodology, there is still some space for improvements. First of all we need to have in mind that albeit numerical simulations are treated as a reliable source, they may still produce some errors. Accuracy of the results of numerical analysis in [10], which was also adapted to our analysis, depends on choice of appropriate amplitude fluctuation, which is chosen



(a) (b)

Fig. 3. Periods of oscillations in the model of Design I.1. according to classical mechanics method (a) and numerical method (b), where $\alpha = 50$, $\Delta = 4$, $\sigma = 1$, $n = 2$ and $m = 2$. White colour denotes stationary behaviour.



(a) (b)

Fig. 4. Periods of oscillations in the model of Design III according to classical mechanics method (a) and numerical method (b), where $\alpha = 50$, $\Delta = 1$, $\sigma = 1$, $n = 3$ and $m = 2$. White colour denotes stationary behaviour.

manually (according to supplementary code in [10] minimal amplitude equals 10^{-5}). It is possible that smaller values would lead to the expansion of oscillatory regions. On the other hand, our approach may also introduce some errors in the characterisation of oscillatory behaviour due to the linearisation of a non-linear dynamical system.

Oscillatory behaviour is, however, also characterized by oscillation amplitudes, which are unfortunately not possible to estimate with the proposed method. Oscillation amplitudes can be, on the other hand, determined with numerical simulations. These can be conducted in a combination with proposed method, which can drastically reduce the parameter space on which numerical simulations are performed (i.e. simulations need to be performed only for parameter values that result in oscillatory behaviour with certain oscillation periods).

We end this discussion with some concrete biological applications of proposed methodology. These can be found in synthetic biology, i.e. for guiding the design of novel biological oscillators, as well as in systems biology, i.e. for the analysis of existing biological oscillators. For example, one can apply the proposed methodology to efficiently analyse different oscillator topologies before their experimental implementation. Methodology is able to efficiently identify the most robust topologies that also reflect desired oscillation periods (in our case the most robust topology, i.e. the one with the largest oscillatory region, is Design I.1). Moreover, analysis conducted with proposed methodology can guide the experimentalist to choose the components (e.g., promoters, ribosome binding sites, protein coding sequences, etc.) with kinetic properties that match the parameter values for which the desired behaviour is obtained. Last but not least, methodology can be applied in the analysis of existing biological oscillators. For example, it can be used as a method behind the parameter estimation techniques to efficiently tune the modelled dynamics with experimental results describing the time-course of concentrations of oscillatory species in the system under study.

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CLASSICAL MECHANICS APPROACH APPLIED TO ANALYSIS OF GENETIC OSCILLATORS

Supplementary material

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1 Models derivation

All the models described presume that the protein binding and mRNA dynamics are much faster than the protein translation and degradation and were obtained in the same way as described in [1, 2]. We give a more detailed derivation of these models in the following sections.

1.1 Design I

Design I presents the topology, in which activator and repressor compete for the same binding sites on the activator promoter. They bind to the promoter in multimerised form and multimerisation can be considered as a single step reaction with the Hill aproximation. Binding and multimerisation reactions can be thus described as follows:

$$P_R + A_n \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P_R A_n,$$

$$P_A + R_m \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P_A R_m,$$

$$P_A + A_n \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} P_A A_n.$$

$$mR \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} R_m,$$

$$nA \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} A_n,$$

Here R represents repressor protein, R_m repressor multimer, A activator protein, A_n activator multimer, P_R repressor promoter, $P_R A_n$ activated repressor promoter, P_A activator promoter, $P_A R_m$ repressed activator promoter and $P_A A_n$ activated activator promoter.

Transcription, translation and degradation reactions can be described in the following way:

$$P_R \overset{\beta_R}{\rightarrow} P_R + m_R,$$

$$P_R A_n \overset{\alpha\beta_R}{\rightarrow} P_R A_n + m_R,$$

$$P_A \overset{\beta_A}{\rightarrow} P_A + m_A,$$

$$P_A A_n \overset{\alpha\beta_A}{\rightarrow} P_A A_n + m_A,$$

$$m_R \overset{\gamma_R}{\rightarrow} m_R + R,$$

$$m_A \overset{\gamma_A}{\rightarrow} m_A + A,$$

$$m_R \overset{\alpha_R}{\rightarrow} 0,$$

$$m_A \overset{\alpha_A}{\rightarrow} 0,$$

$$R \overset{\delta_R}{\rightarrow} 0,$$

$$A \overset{\delta_A}{\rightarrow} 0.$$

Here m_R represents repressor mRNA and m_A represents activator mRNA.

Since binding and multimerisation reactions occur in a much faster timescale than transcription, translation and degradation, they can be assumed to be in equilibrium.

Repressor promoter can be in two different states in Design I topology, i.e. unoccupied (P_R) or activated ($P_R A_n$). Weights of these states can be expressed in the following way:

$$W(P_R) = 1,$$

$$W(P_R A_n) = K_1 K_A A^n,$$

where A denotes the concentration of activator protein, $K_1 = \frac{k_1}{k_{-1}}$ and $K_A = \frac{k_6}{k_{-6}}$.

Probability of each promoter state can be calculated with the fractional occupancy approach [3] applying the weights in the following equation:

$$p(s_i) = \frac{W(s_i)}{\sum_{j=1}^{k} W(s_j)},$$

where k is the number of all possible states. In our case probabilities are

$$p(P_R) = \frac{1}{1 + K_1 K_A A^n},$$

$$p(P_R A_n) = \frac{K_1 K_A A^n}{1 + K_1 K_A A^n}.$$

The rate of repressor mRNA transcription thus equals

$$P_R^T \beta_R \frac{1 + \alpha K_1 K_A A^n}{1 + K_1 K_A A^n},$$

where P_R^T is the total concentration of repressor promoter.

Transcription rate of activator mRNA can be expressed in a similar way. Activator promoter can be in three different states in Design I topology, i.e. unoccupied (P_A), repressed ($P_A R_m$) or activated ($P_A A_n$). Weights of these states can be expressed as

$$W(P_A) = 1,$$

$$W(P_A R_m) = K_2 K_R R^m,$$

$$W(P_A A_n) = K_3 K_A A^n,$$

where R denotes the concentration of repressor protein, $K_2 = \frac{k_2}{k_{-2}}$, $K_3 = \frac{k_3}{k_{-3}}$ and $K_R = \frac{k_5}{k_{-5}}$. State probabilities are thus

$$p(P_A) = \frac{1}{1 + K_2 K_R R^m + K_3 K_A A^n},$$

$$p(P_A R_m) = \frac{K_2 K_R R^m}{1 + K_2 K_R R^m + K_3 K_A A^n},$$

$$p(P_A A_n) = \frac{K_3 K_A A^n}{1 + K_2 K_R R^m + K_3 K_A A^n}$$

and transcription rate of activator mRNA

$$P_A^T \beta_A \frac{1 + \alpha K_3 K_A A^n}{1 + K_2 K_R R^m + K_3 K_A A^n},$$

where P_A^T is the total concentration of activator promoter.

Combining the transcription rates with the mRNA degradation yields the following differential equations governing the dynamics of mRNA species:

$$\frac{dm_R}{dt} = \beta_R P_R^T \frac{1 + \alpha K_1 K_A A^n}{1 + K_1 K_A A^n} - \alpha_R m_R,$$

$$\frac{dm_A}{dt} = \beta_A P_A^T \frac{1 + \alpha K_3 K_A A^n}{1 + K_2 K_R R^m + K_3 K_A A^n} - \alpha_A m_A,$$

where m_R and m_A denote the concentrations of repressor and activator mRNA. Activator and repressor dynamics governed by translation and degradation can be described with two additional differential equations:

$$\frac{dR}{dt} = \gamma_R m_R - \delta_R R,$$

$$\frac{dA}{dt} = \gamma_A m_A - \delta_A A.$$

These equations can be simplified with the quasi-steady-state approximation, i.e. $\frac{dm_R}{dt} \approx 0$ and $\frac{dm_a}{dt} \approx 0$ [1], which yields the following model:

$$\frac{dR}{dt} = \gamma_R \frac{\beta_R}{\alpha_R} P_R^T \frac{1 + \alpha K_1 K_A A^n}{1 + K_1 K_A A^n} - \delta_R R,$$

$$\frac{dA}{dt} = \gamma_A \frac{\beta_A}{\alpha_A} P_A^T \frac{1 + \alpha K_3 K_A A^n}{1 + K_2 K_R R^m + K_3 K_A A^n} - \delta_A A.$$

1.2 Design I.1

Variation of Design I (Design I.1) includes a binding site for repressor on its own promoter. It can be modelled with an additional state of repressor promoter, i.e. $P_R R_m$, for which the transcription rate is assumed to be zero. The rate of repressor mRNA transcription thus equals

$$P_R^T \beta_R \frac{1 + \alpha K_1 K_A A^n}{1 + K_1 K_A A^n + K_4 K_R R^m},$$

where $K_4 = \frac{k_4}{k_{-4}}$, and k_4 and k_{-4} describe the on-rate and the off-rate constants of the repressor and its promoter binding. The rest of the model can be derived in the same way as for Design I and can be described as

$$\frac{dR}{dt} = \gamma_R \frac{\beta_R}{\alpha_R} P_R^T \frac{1 + \alpha K_1 K_A A^n}{1 + K_1 K_A A^n + K_4 K_R R^m} - \delta_R R,$$

$$\frac{dA}{dt} = \gamma_A \frac{\beta_A}{\alpha_A} P_A^T \frac{1 + \alpha K_3 K_A A^n}{1 + K_2 K_R R^m + K_3 K_A A^n} - \delta_A A.$$

1.3 Design III

Design III implementation includes separate binding sites for activator and repressor on the activator promoter. The adaptation of Design I to Design III model is again straightforward. We need to regard additional state of activator promoter, i.e. $P_A R_m A_n$, for which the transcription rate is again assumed to be zero. The rate of activator mRNA transcription thus equals:

$$P_A^T \frac{1 + \alpha K_3 K_A A^n}{1 + K_2 K_R R^m + K_3 K_A A^n + K_2 K_R R^m K_3 K_A A^n}.$$

Again, the same procedure as in Design I can be followed in the rest of the derivation. It brings us to the following model:

$$\frac{dR}{dt} = \gamma_R \frac{\beta_R}{\alpha_R} P_R^T \frac{1 + \alpha K_1 K_A A^n}{1 + K_1 K_A A^n} - \delta_R R,$$

$$\frac{dA}{dt} = \gamma_A \frac{\beta_A}{\alpha_A} P_A^T \frac{1 + \alpha K_3 K_A A^n}{1 + K_2 K_R R^m + K_3 K_A A^n + K_2 K_R R^m K_3 K_A A^n} - \delta_A A.$$

1.4 Rescaling the variables

Transformation to the models presented in the main text can be achieved by rescaling all variables to become dimensionless [1, 2]:

$$\begin{aligned}
x &= \sqrt[n]{K_3 K_A} A, \\
y &= \sqrt[m]{K_3 K_A} R, \\
\tau &= t\delta_R, \\
\Delta &= \frac{\delta_A}{\delta_R}, \\
\beta &= \frac{\gamma_A \beta_A}{\delta_A \alpha_A} P_A^T \sqrt[n]{K_3 K_A}, \\
\gamma &= \frac{\gamma_R \beta_R}{\delta_R \alpha_R} P_R^T \sqrt[m]{K_3 K_A}, \\
\sigma &= \frac{K_2 K_R}{K_3 K_A}.
\end{aligned}$$

1.5 Parameter values

The nominal parameter values used in our analysis were obtained from the literature [1, 2] and are as follows:

- $m = n = 2$,
- $K_1 K_A = K_2 K_R = 10^{-3} n M^{-1}$,
- $K_1 = K_3$,
- $K_2 = K_4$,
- $K_A = K_R$,
- $\beta_A = \beta_R = 10 h^{-1}$,
- $\alpha_A = \alpha_R = 5 h^{-1}$,
- $\alpha = 50$,
- $\gamma_A = 50 \cdot \frac{\alpha_A}{\beta_A}$,

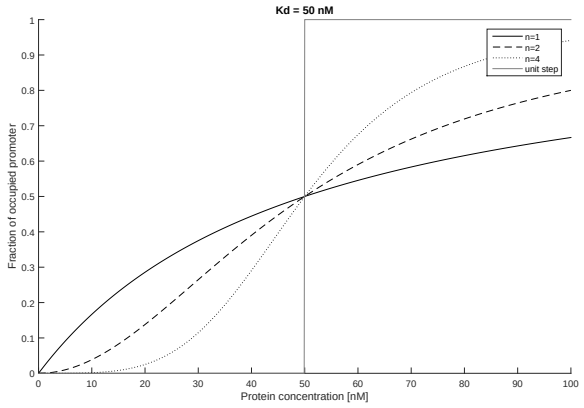

Figure 1: Comparison of the non-linearity of promoter response approximation between biological plausible scenarios (e.g., n=1, n=2 or n=4) and approach using Heaviside or unit-step function.

- $\gamma_R = 0.1 \cdot \gamma_A$,
- $\delta_A = 1h^{-1}$,
- $\delta_R = 0.1h^{-1}$,
- $P_A^T = P_R^T = 1$.

2 Using unit step function to approximate promoter activity

Approaches described in [4] and [5] rely on the approximation of a promoter activity with a Boolean variable with only two possible values, i.e. zero (not active) and one (active), using unit step function as $\theta\left(A - K_{d_A}\right)$ for activation and $\theta\left(K_{d_R} - R\right)$ for repression. Here K_{d_A} and K_{d_R} present the dissociation constants that can be interpreted as transcription factor concentrations producing half occupation of the promoter. This approximations yield a very high non-linearity of promoter response, which is often not plausible. For example, the model we used in our case study presumes the Hill coefficients equal to 2 or 3, which generates a response that is far from unit step function (see Fig. 1).

We can be still apply this approximation to the amplified negative feedback oscillator models described in previous section. In this case dissociation

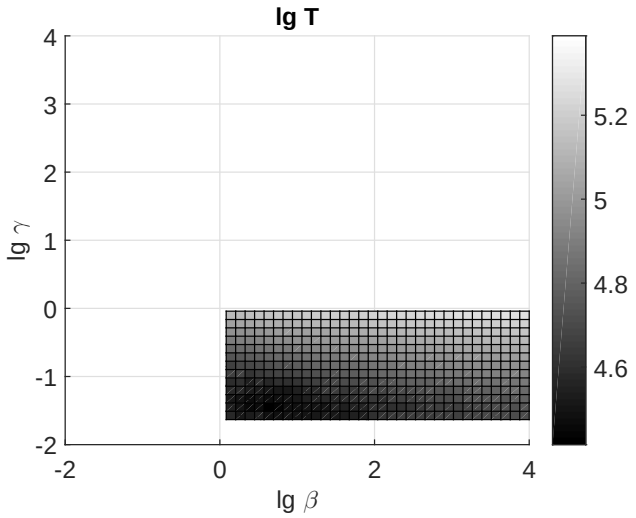

Figure 2: Oscillation periods obtained on the unit step function model of Design I and Design III implementation of an amplified negative feedback oscillator.

constants can be expressed as

$$K_{d_A} = \sqrt[n]{\frac{1}{K_1 K_A}},$$
$$K_{d_R} = \sqrt[m]{\frac{1}{K_2 K_R}}.$$

For example, Design I and Design III models can be adapted to

$$\frac{dR}{dt} = \gamma_R \frac{\beta_R}{\alpha_R} P_R^T \left(\theta\left(K_{d_A} - A\right) + \alpha\theta\left(A - K_{d_A}\right)\right) - \delta_R R,$$

$$\frac{dA}{dt} = \gamma_A \frac{\beta_A}{\alpha_A} P_A^T \theta\left(K_{d_R} - R\right)\left(\theta\left(K_{d_A} - A\right) + \alpha\theta\left(A - K_{d_A}\right)\right) - \delta_A A.$$

Note that this modelling approach is due to the simplification with a unit step function unable to differ among Design I and Design III implementation. Results of previous analyses however indicate that substantial differences in the dynamics of these two topologies exist. We performed the analysis on the simplified model with the same parameter values as described in the main text. Model yielded solutions that were hardly comparable to the results of non-simplified models (see Fig. 2). We can conclude that the unit step function approximation can be applied only to a limited scope of biological systems and has a very limited biological relevance.

3 Calculation of the LCM of real numbers

Here we demonstrate that the LCM of two numbers A and B can be obtained from the formula

$$LCM(A,B) = \frac{LCM(m_1,m_2)}{GCF(n_1,n_2)}, \tag{1}$$

where GCM stands for the greatest common multiplier. If A and B are rational numbers (according to limited numerical accuracy we are always dealing with rational numbers in computational calculations) it means that they can be expressed in the form $A = m_1/n_1$ and $B = m_2/n_2$, which brings us to relation

$$\begin{aligned} \frac{LCM(A,B)}{A} &= \frac{LCM(m_1,m_2)}{m_1} \cdot \frac{n_1}{GCF(n_1,n_2)} \\ &= \text{integer} \cdot \text{integer} = \text{integer}. \end{aligned} \tag{2}$$

4 Evaluating the threshold values

Oscillatory behavior is sometimes achieved also with non-zero imaginary parts of calculated circular frequencies, which should be however substantially smaller than real parts. Threshold values that we use in the analysis should minimize the errors of our approach in comparison to a credible source of data, e.g., numerical simulations, on a testing set of parameter values. Percentage error can be quantified with the following measure:

$$E = \frac{n_c}{n_T} \cdot 100\%, \tag{3}$$

where n_c presents the number of conflict points and n_T the total number of points on which evaluation is performed. Note that the denominator uses the total number of points, while even numerical simulations cannot be considered as an absolutely accurate method. Fig. 3 presents the values of E in dependence on the threshold value for Design I. Threshold value in which E is minimized was used for period estimation in the main paper. The same value can be used on all three models or even in a general case. One can alternatively determine specific threshold values that are optimal for a given model to obtain more accurate results.

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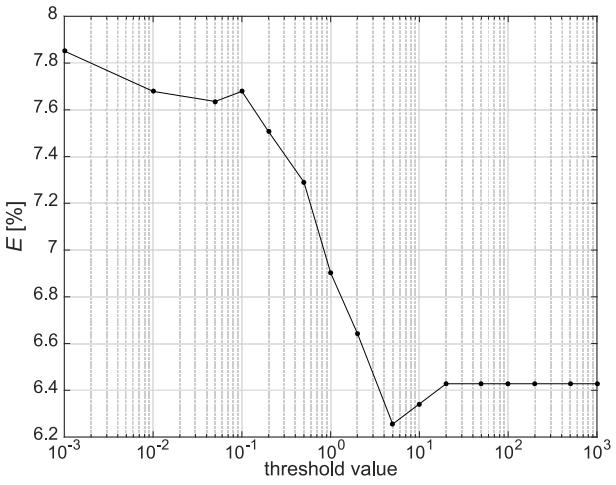

Figure 3: Effect of threshold values on the error introduced by classical mechanics approach in comparison to numerical simulations for Design I model.

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