Phase tracking and restoration of circadian rhythms by model-based optimal control

O. S. SHAIK, S. SAGER, O. SLABY, D. LEBIEDZ*
Interdisciplinary Center for Scientific Computing,
University of Heidelberg, Germany

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Abstract

Periodic cellular processes and especially circadian rhythms, governed by the oscillating expression of a set of genes based on feedback regulation by their products have become an important issue in biology and medicine. The central circadian clock is an autonomous biochemical oscillator with a period close to 24 hours. Research in chronobiology demonstrated that light stimuli can be used to delay or advance the phase of the oscillator, allowing it to influence underlying physiological processes. Phase shifting and restoration of altered rhythms can generally be viewed as open-loop control problems that may be used for therapeutic purposes in diseases. We study a circadian oscillator model of the central clock mechanism for the fruit fly *Drosophila* and show how model-based mixed-integer optimal control allows for the design of chronomodulated pulse-stimuli schemes achieving circadian rhythm restoration in mutants and optimal phase synchronization between the clock and its environment.

Keywords: Circadian clock; mixed-integer optimal control; bang-bang control; phase tracking and entrainment

1 Introduction

Self-organized rhythmic processes are encountered at all levels in cell biology and are a subject of great interest for both biological and mathematical research communities [1]. Biological systems are open and kept far from equilibrium by fluxes of matter and energy. For these reasons, the systems are always exposed to external perturbations and the balance between robustness and sensitivity to external stimuli is a crucial issue.

*Corresponding author. E-Mail address: lebiedz@iwr.uni-heidelberg.de

The role of circadian rhythms with a period of nearly 24 hours is of particular importance because many physiological and behavioral functions of living creatures, ranging from insects to mammals, appear to be governed by this so called "master clock". A pacemaker delivers a circadian rhythm, generated by periodic activation/inhibition of transcription of a set of genes, denoted as "clock genes". The molecular basis of these mechanisms has been clarified over the past decade, first for an insect, the fruit fly *Drosophila* [2] and more recently also for mammals (see [3] for a review). Circadian rhythms represent one of the most intensely studied examples of oscillatory systems in biology and a variety of mathematical models has been developed which accurately describe many properties of circadian rhythms [31].

The central mechanism of circadian clocks seems to be conserved among many organisms and is based on a feedback regulated gene transcription network in the cell nucleus and its corresponding protein translation products in the cytoplasm. Disorders of the circadian system and of the circadian pacemaker interfere with the timing of sleep and waking and can affect sleep onset, duration, sleep quality and sleep episode duration. Misalignment between the internal circadian pacemaker and the external environment is believed to cause health problems such as cardiovascular disease, diabetes, sleep disorders, and gastro-intestinal disorders [4, 5]. The external natural light-dark cycle is the primary environmental stimulus for entraining circadian rhythms in most species, including humans. In fact, some of the most intriguing observations related to circadian rhythms are that they are entrained by periodic light and darkness periods, persist under conditions of complete darkness and can be modified (phase shifted) by external light stimuli.

Taking into account these issues, medical techniques known as "chronotherapy" have been developed over the past twenty years [6] and mathematical models can be exploited for these tasks [9]. Chronotherapy can be viewed as a therapeutic control operated via drug injection schedules or modifications of the environment (e.g., exposure to light or feeding). Cancer is one field of medicine where chronotherapeutic approaches have been developed and tested [10]. Chronomodulated injection for example allows to lower considerably the side effects of highly toxic anticancer drugs [7] in chemotherapy.

Clinical observations indicate that circadian rhythms may be altered in many types of cancer [11]. It has also very recently been established that the circadian clock plays a key role in tumor suppression [12] and that rhythm alteration itself might even cause cancer. Thus an additional goal of cancer therapy beyond the destruction of tumor cells might be the restoration of the endogenous circadian time structure because such a restoration could improve the prospects of patient recovery [8].

All these aspects and considerations motivate the study of control problems devoted to targeted manipulation of circadian rhythms. Mathematical models are a valuable tool here in order to analyze potential control schemes. A general problem formulation may be to act upon the central clock system considered in a pathological state in order to modify its properties and bring it back close to a desired target state, e.g. the healthy state.

In order to demonstrate exemplarily the value of mathematical models and the application of advanced numerical control techniques, we study rhythm control schemes of the central clock genes and proteins for the fruit fly *Drosophila*. A simplified *Drosophila* model has recently been studied from a control point of view in [13], where the authors theoretically investigate periodic activation/inhibition schemes of the translation frequency of messenger RNA of a clock gene. Flatness based control methods have been applied to control protein concentration oscillations in [14]. The aim was to restore a circadian rhythm of mutants showing either too short or too long periodicity of their endogenous cycle. Careful analysis of the effects of a single short exposure to bright light or of interrupted bright light pulses may have important implications for the practical application of light treatment in case of circadian rhythm sleep disorders. Compared with constant stimulation or once-a-day administration, which are both inefficient, intermittent stimulation of several hours duration within a 24 hours period is successful in achieving control aims in the *Drosophila* model [13]. However, repetitive uninterrupted exposure to bright light for many hours and days is often not feasible in a clinical settings.

Here, we use model-based optimal control of mixed-integer type for systematically finding appropriate external pulse-stimuli leading to optimal synchronization of the circadian model with the desired behavior like specific phase shifting or restoration of altered rhythms of mutants. Possible control parameters are the rates of protein synthesis and degradation. Our integer optimal control approach allows to systematically identify stimuli that switch between two given bounds for the control parameter (bang-bang control). Such discrete pulse-stimuli can be much more easily realised in practice than continously varying control functions. However, the numerical solution of mixed-integer problems is extremely challenging and we apply a recently developed powerful algorithm based on multiple shooting (see section 4).

1.1 Model

One of the detailed models available for the circadian clock is based on experimental observations collected for the fruit fly *Drosophila*, a widely used model organism in biology. The model [22] (schematized in figure 1) is centered around negative auto-regulation of gene expression. It takes into account nuclear transcription of the *per* and *tim* genes and transport of the *per* and *tim* mRNAs into the cytoplasm, where they are translated into PER and TIM proteins. The latter can be multiply phosphorylated and form a complex that enters the nucleus and represses *per* and *tim* transcription. The model incorporates degradation of the PER and TIM proteins and their mRNAs. Light entrainment necessitates modeling the transcriptional regulation of both key proteins PER and TIM, since light selectively promotes the degradation of TIM [26, 27]. The maximum rate of TIM degradation ν_{dT} increases with increasing light intensity, where a 10-min light pulse is assumed to double the rate constant for a duration of 3h [22]. In mammals, where *per* and *tim* genes are also found, light acts by enhancing the rate of *per* expression ν_{sP} [16]. The *Drosophila* model is described by a

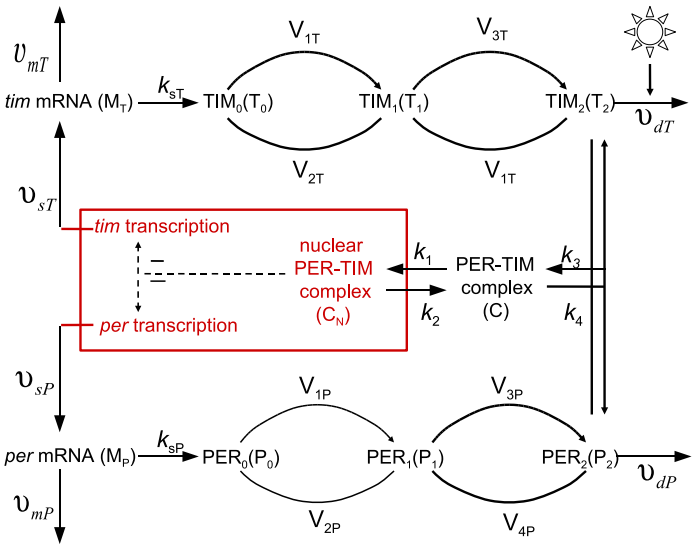

Figure 1: Model for circadian oscillator in *Drosophila* involving negative regulation of gene expression by PER and TIM. *per* (M_P) and *tim* (M_T) mRNAs are synthesized in the nucleus and transferred into the cytoplasm, where they accumulate at the maximum rates ν_{sP} and ν_{sT}, respectively. There, they are degraded enzymatically at the maximum rates, ν_{mP} and ν_{mT}, with the Michaelis-Menten constants, K_{mP} and K_{mT}. The rates of synthesis of the PER and TIM proteins are proportional to M_P and M_T characterized by the apparent first-order rate constants k_{sP} and k_{sT}. Parameters $V_{iP}(V_{iT})$ and $K_{iP}(K_{iT})$ (i = 1,...4) denote the maximum rate and Michaelis constant of the kinase and phosphatases involved in the reversible phosphorylation of P_0 (T_0) into P_1 (T_1) and P_1 (T_1) into P_2 (T_2), respectively. The fully phosphorylated forms (P_2 and T_2) are degraded by enzymes with maximum rate ν_{dP} and ν_{dT} and Michaelis-Menten constants K_{dP} and K_{dT} and reversibly form a complex C (association and dissociation are characterized by the rate constants k_3 and k_4), which is transported into the nucleus at a rate characterized by the apparent first-order rate constant k_1. Transport of the nuclear form of the PER-TIM complex (C_N) into the cytoplasm is described by the apparent first-order rate constant k_2. The negative feedback exerted by the nuclear PER-TIM complex on *per* and *tim* transcription is modeled by a Hill-type equation. For the full kinetic model equations see [22].

set of 10 ordinary differential equations (ODEs) that govern the time evolution of the concentrations of *per* and *tim* mRNAs and of the various forms of PER and TIM proteins and the PER-TIM complex [22] with 38 model parameters. The model can reproduce circadian oscillations in continuous darkness, entrain-

ment by light-dark cycles, and phase shifting by light pulses. Figure 2 shows the oscillations in total PER protein (P_t) level, per mRNA (M_p), and nuclear PER-TIM complex (C_N) under dark conditions. Such conditions are accounted for in the *Drosophila* model by holding the parameter, which measures the maximum rate of TIM degradation $\nu_{dT} = 2.4nMh^{-1}$ at a constant low value. The PER-TIM control system generates autonomous oscillations with a period of 24 hours for the set of parameter values considered in Table 1.

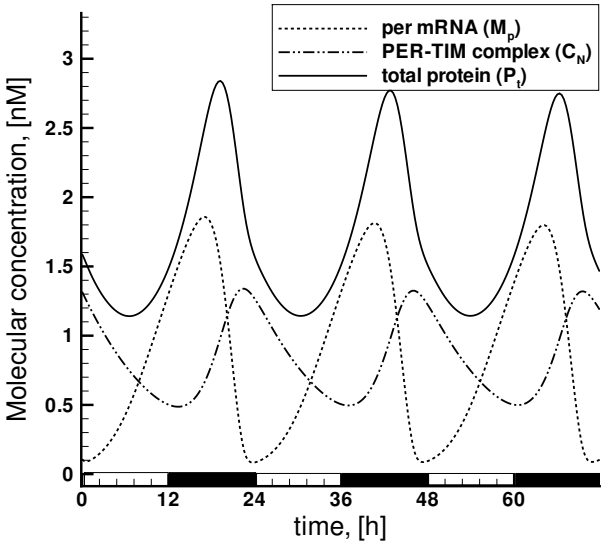

Figure 2: Oscillations of circadian rhythms in *Drosophila* for the model provided in Table 1 under continuous darkness. The curves are obtained by numerical integration using the BDF-Integration formula implemented in DAESOL-II [17].

The full model equations are given in appendix A. The parameters with comparably strong phase and period effects control the degradation of mRNAs and proteins and the protein translation. Using this knowledge, it is in principle possible to modify the related processes in a desired way. For example, transcription rates can be varied by tuning the promoter strength using directed evolution [28], translation rates are influenced by ribosomal binding sites of different activities [29], and degradation kinetics of mRNA can be altered through modification of its secondary structure for stability [30].

We use the model of circadian rhythms in *Drosophila* [22] for our optimal control study. After a short analysis of its bifurcation structure, for a better understanding of the underlying dynamics and its dependence on external stimuli, we want to demonstrate the control possibilities in two different optimal control tasks. In the first scenario, we look at the so called "phase-tracking", where

we compute stimuli, which cause a phase-shift of the oscillation-period and in the second scenario we demonstrate how to control the restoration of a normal circadian cycle for mutant type *Drosophila*, which have a non 24-hours period by controlling the rate of protein synthesis.
The results may be useful in the treatment of circadian rhythm disorders, such as delayed-sleep phase syndrome, advanced-sleep phase syndrome, shift-work dyssomnia and jet-lag while improving alertness and performance through synchronizing the biological clock with its environment [15] and may be useful in cancer chronotherapy when circadian rhythms are thought to be altered.

2 Bifurcation Analysis

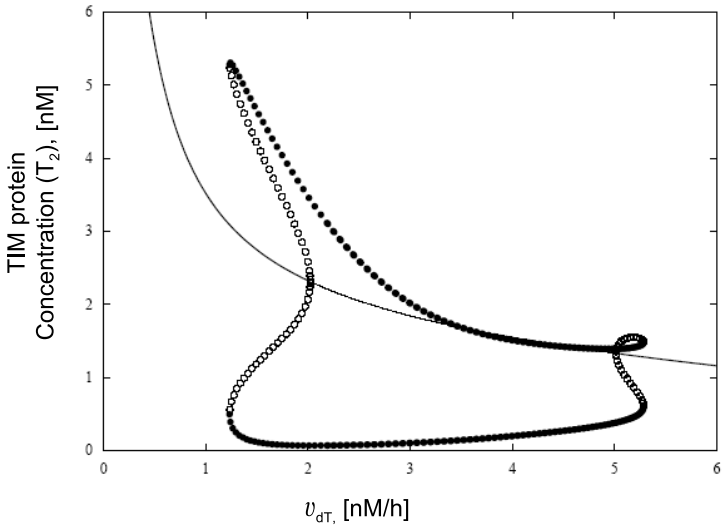

Figure 3: Bifurcation diagram showing the range of sustained oscillations as a function of the light-controlled parameter in the molecular model for the circadian clock [22]. The diagram represents the stable (solid line) or unstable (dashed line) steady-state value of a state variable (the concentration of the phosphorylated TIM form), as well as the envelope (maximum and minimum oscillation value) of stable (solid circles) or unstable (open circles) sustained oscillations, as a function of ν_{dT}. Numerical computations for the bifurcation diagram were performed with the software package AUTO [33].

For a better understanding of the results of our optimal control approach, we use a bifurcation analysis of the model for the circadian clock with respect to the light-sensitive parameter ν_{dT}, which is shown in the figure 3. It represents the dynamic behavior of the oscillatory system by a single state variable, the

fully phosphorylated form of the TIM protein (T_2), as a function of ν_{dT}. At low values of ν_{dT}, a stable steady state is obtained. As ν_{dT} increases, the steady state becomes unstable, and sustained limit cycle oscillations occur. Beyond a second bifurcation point at large ν_{dT}, the steady state recovers its stability. For some parameter values, coexistence of a stable steady state and a stable regime of limit cycle oscillations is observed.

In this situation of coexisting stable steady states and stable limit cycle oscillations, the effect of a light pulse can bring the oscillating system into the basin of attraction of the stable singularity so that circadian oscillations are suppressed permanently. The duration and amplitude of the light-induced biochemical changes, that succeed in suppressing the rhythm, vary with the phase of the rhythm when the light pulse is applied. A second light pulse can bring the system back from the stable steady state into the basin of attraction of the stable oscillations. A recent analysis for finding the optimal light stimulus by mixed-integer optimization to permanently suppress and restore the circadian rhythms has been studied [18]. In general, a finite stimulus will force a deviation of the oscillator's trajectory but it will return to stable limit cycle asymptotically. However, the system undergoes a phase shift and depending upon the time and strength of the stimulus the resultant phase will vary. In the following, we will exploit the described occurrence of a stable steady state and a limit cycle to systematically compute mixed-integer optimal controls, which drive the dynamics into the region of the stable steady state and back to achieve, for example, a specific desired phase shift for a mutant type *Drosophila* with non 24-hours period circadian rhythms.

3 Formulation of optimal control problems

The aim of our approach is to change the behavior of the dynamical system by a time variant external control such as the maximum rate of protein degradation (ν_{dT}) or translational frequency (k_s). We will denote the corresponding functions by $u(t)$. We want to minimize the integrated difference between the state trajectory $x(t)$ of the system and a reference trajectory $x_r(t)$. This reference trajectory is obtained by solving a boundary value problem that includes a periodicity constraint

$$x_r(0) = x_r(T)$$

with $T = 24$. We fix the parameters to the values given in Table 1, which corresponds to a "darkness scenario" except ν_{dT}. We apply the optimal control software package MUSCOD-II [21] that implements the direct multiple shooting method, see section 4.1. As a numerical solution of the boundary value problem, we obtain the value of the parameter $\nu_{dT} = 2.4nMh^{-1}$ for which the system shows the desired periodic behavior with a period of exactly 24 hours. As can be seen in figure 3, for the value $\nu_{dT} = 2.4nMh^{-1}$ the system is characterized by an unstable steady state surrounded by stable limit cycle oscillations respresented

in solid circles. Therefore, the calculated reference orbit $x_r(t)$ corresponding to $\nu_{\mathrm{dT}} = 2.4nMh^{-1}$ is stable.

Our optimization problem now consists of minimizing the deviation from this reference trajectory for given initial values $x(0) = x_0$ over a given time horizon,

$$\begin{aligned} \min_{u,x} \quad & \int_0^t \|x(t) - x_r(t)\|_2^2 \mathrm{d}t \\ \text{subject to} \quad & \dot{x} = f(x, u, p), \\ & x(0) = x_0, \\ & x(t) \geq 0, \\ & u_{min} \leq u(t) \leq u_{max}. \end{aligned} \tag{1}$$

The constraints of the optimization problem are the function f, which represents the differential equation model given in appendix A, control boundaries $u \in [u_{\min}, u_{\max}]$ and positive concentrations.

In the first control scenario, for phase tracking of circadian rhythms, we use the light sensitive ν_{dT} as a control parameter and the other parameter values are fixed to the values given in Table 1. In the second control scenario, for restoration of altered rhythms, we use the translation frequency (k_s) of the proteins PER and TIM as a control parameter, which may be influenced by suitable drugs.

In practice, the light sensitive parameter ν_{dT}, and the translation frequency k_s are easier to control as a switching off-on-off control function than a function with continous control values over time. Mathematically this means that we have to restrict the control function $u(t)$ to take values in $\{u_{\min}, u_{\max}\}$ only. This can be reformulated via $u(t) = u_{\min} + w(t)(u_{\max} - u_{\min})$ into a binary valued control function $w(t) \in \{0, 1\}$.

4 Numerical Optimal Control Methods

4.1 Direct Methods of Optimal Control

There are various methods in the literature to solve optimal control problems for ODE. We choose Bock's direct multiple shooting method, [23], as this approach has proven to be a reliable tool not only for mechanics and chemical engineering, but also in systems biology of self-organization, e.g., [19], [20]. It is a direct method and therefore based on a transformation of the infinite–dimensional control problem to a finite–dimensional nonlinear program (NLP) by a discretization of the control functions. A time grid of *multiple shooting nodes* is introduced,

$$0 \leq t_1 \leq \ldots \leq t_{n_{\mathrm{ms}}} = T. \tag{2}$$

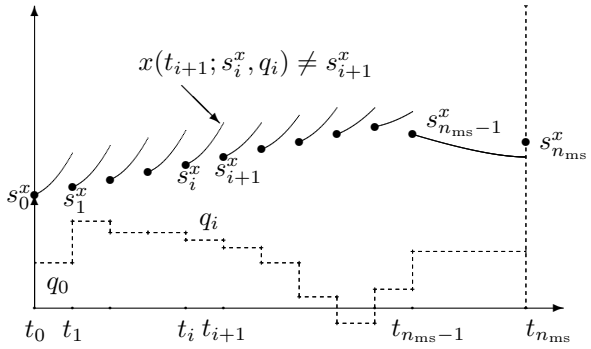

Figure 4: Illustration of direct multiple shooting during SQP iterations. The controls are discretized, the corresponding states obtained by piecewise integration. The matching conditions are violated in this scheme — the overall trajectory is not yet continuous.

With finitely many control parameters $q_i \in \mathbb{R}^{n_i}$,

$$q = (q_0, q_1, \ldots, q_{n_{\rm ms}-1})^T,$$

a piecewise approximation $\hat{u}$ of the control functions u on the grid (2) is then defined by

$$\hat{u}(t) \quad = \quad \varphi_i(t, q_i), \quad t \in [t_i, t_{i+1}], \quad i = 0, \ldots, n_{\rm ms} - 1. \tag{3}$$

In practice the functions φ_i are typically vectors of constant, linear or cubic functions. On the grid (2) node values $s_i^x \approx x(t_i) \in \mathbb{R}^{n_x}$ are introduced, from now on $0 \leq i < n_{\rm ms}$, that serve as initial values of intermediate trajectories. All values $x(t)$ in between the grid points are obtained by a decoupled integration with an ODE solver on each of the multiple shooting intervals. Continuity of the state trajectory at the multiple shooting grid points

$$s_{i+1}^x \quad = \quad x(t_{i+1}; s_i^x, q_i, p) \tag{4}$$

is incorporated via equality constraints into the NLP. Here $x(\cdot)$ denotes the solution of the ODE on interval $[t_i, t_{i+1}]$ with initial values s_i^x at time t_i. Figure 4 illustrates the concept of direct multiple shooting. The control variables q_i, the global parameters p, that may include the time horizon length $h = t_f - t_0$ for problems with free end time, and the node values s_i^x are the degrees of freedom of the discretize and parameterized optimal control problem. If we write them in one n_ξ–dimensional vector

$$\xi \quad = \quad (s_0^x, q_0, s_1^x, \ldots, q_{n_{\rm ms}-1}, s_{n_{\rm ms}}^x, p)^T, \tag{5}$$

subsuming all equality constraints and continuity conditions (4) in a function $G(\xi)$ and all inequality constraints in a function $H(\xi)$ the resulting NLP can be

formulated as

$$\begin{aligned} \min_{\xi} \quad & F(\xi) \\ \text{subject to} \quad & G(\xi) = 0, \\ & H(\xi) \leq 0. \end{aligned} \tag{6}$$

This NLP can be solved with tailored iterative methods exploiting the structure of the problem, e.g., by sequential quadratic programming (SQP). The continuity conditions do not necessarily have to be satisfied during the iterations of the SQP algorithm used to solve the NLP, but surely when convergence has been achieved. Direct multiple shooting is therefore a so called all–at–once approach that solves the dynamic equations and the optimization problem at the same time opposed to the sequential approach of single shooting that computes a continuous trajectory as a feasible ODE solution in every iteration. For more details on direct multiple shooting, see [23] or [21].

4.2 Mixed-Integer Optimal Control Methods

If the optimal control problem under consideration contains control functions $w(\cdot)$ with a restriction to values in a disjoint set, say to $\{0,1\}^{n_w}$, the methods have to be extended. We say that a trajectory $\mathcal{T} = (x, w, u, p)$ is binary admissible, if all constraints are fulfilled and $w(t) \in \{0,1\}^{n_w}$ for all $t \in [t_0, t_f]$. For the application treated in this paper, we apply the novel algorithm MSMINTOC introduced in [24] that can be sketched as follows. We relax the control functions to $w(\cdot) \in [0,1]^{n_w}$. We solve the relaxed problem for a given control discretization $\mathcal{G}^0$ and obtain the grid–dependent optimal function value $\Phi^{\mathrm{RL}}_{\mathcal{G}^0}$. We iterate on a refinement of the grid for n_{ext} steps with the idea to extrapolate towards $n_{\mathrm{ms}} \mapsto \infty$. We obtain $\Phi^{\mathrm{RL}} = \Phi^{\mathrm{RL}}_{\mathcal{G}^{n_{\mathrm{ext}}}}$ as the objectivefunction value on the finest grid $\mathcal{G}^{n_{\mathrm{ext}}}$. This objective function value serves as a lower bound that can be approximated up to any user–specified tolerance $\varepsilon > 0$ by a binary admissible trajectory, for a proof see [24]. If the optimal trajectory on $\mathcal{G}^{n_{\mathrm{ext}}}$ is already binary admissible then stop. Otherwise apply a rounding or penalty heuristics on the grid. If the trajectory is binary admissible, obtain an upper bound Φ^{ROU}. If $\Phi^{ROU} < \Phi^{\mathrm{RL}} + \varepsilon$ then stop. Otherwise optimize the switching times for a fixed switching structure, initialized with the trajectory obtained by heuristics. Again, if the obtained trajectory is binary admissible, obtain an upper bound Φ^{STO} and if $\Phi^{STO} < \Phi^{\mathrm{RL}} + \varepsilon$ then stop. For most practical problems and the model under consideration in this study a modest iteration on n_{ext} is sufficient to obtain a binary admissible trajectory that is within a certain tolerance to the reachable objective function value. If this is not the case, a further interplay between a penalty term homotopy with an adaptive refinement of the control discretization grid or even a rigorous determination of the global solution on a grid by, e.g., Branch & Bound is necessary. See [24] for details, proofs and applications.

5 Results

5.1 Optimal phase tracking of circadian rhythms

Circadian rhythms can be phase shifted by a light pulse. The result can be a phase advance, a phase delay, or no measurable phase change at all [25]. Plotting the direction and magnitude of the phase shift as a function of the phase of the rhythm, when the perturbation is timed, yields the phase-response curve. Molecular models have been used to obtain theoretical phase-response curves that can be compared with experimental observations ([31, 22, 32]).
Here, the aim of our optimal control approach is to automatically identify strength and timing of light-switching induced parameter changes for TIM protein degradation which synchronize the system with a desired reference trajectory and result in an induced phase shift. We use the light-sensitive control parameter $\nu_{\mathrm{dT}}(t)$ as a control function and compute a relaxed optimal control $\nu_{\mathrm{dT}}(t)$ as a solution of problem (1) after relaxation of the integer constraints to $w(t) \in [0, 1]$ using piecewise constant control parameterization. The result for the control function is shown in figure 5 (*left*) and the corresponding controlled system trajectory is shown in figure 6 (*left*). The controller is able to recover a maximum 12-hours phase difference within 40-hours with 0.5 hours accuracy. Obviously the rhythm can be successfully tracked by continuously adjustable time-varying light stimuli.
However, these continuously changing controls are difficult to realize in practice and therefore, we go on to compute a pulse control in terms of a bang-bang solution of problem (1) that switches between a maximal and minimal value of the control parameter. In figure 5 (*right*), the obtained pulse control computed via mixed-integer optimal control and in figure 6 (*right*) the corresponding controlled system state trajectory of PER-TIM protein complex in the nucleus is plotted. From figure 6, it is obvious that there is hardly a difference between the relaxed and the mixed integer result.

5.2 Restoration of altered circadian rhythms

The *Drosophila* circadian rhythm model can also be used for studying possibilities to modify pathological rhythms, e.g. to restore the normal characteristics of the circadian time structure, bearing in mind possible applications in pharmacokinetics. In this case, the aim of our control problem is to determine the type of perturbation by which pathological oscillations could be reverted optimally to the normal pattern of oscillation.

We model altered pathological rhythm by changing the parameters $\nu_{\mathrm{dP}} = 2.4nMh^{-1}$ and $\nu_{\mathrm{dT}} = 2.4nMh^{-1}$, the maximal degradation of PER and TIM proteins. These parameter values represents the nonmutant or "wild-type" *Drosophila*, with an oscillation period of 24 hours. By changing the parameter values to $\nu_{\mathrm{dP}} = 4.5nMh^{-1}$ and $\nu_{\mathrm{dT}} = 4.5nMh^{-1}$, we model the mutant *Drosophila*, called per^l, and with $\nu_{\mathrm{dP}} = 1.25nMh^{-1}$ and $\nu_{\mathrm{dT}} = 1.25nMh^{-1}$,

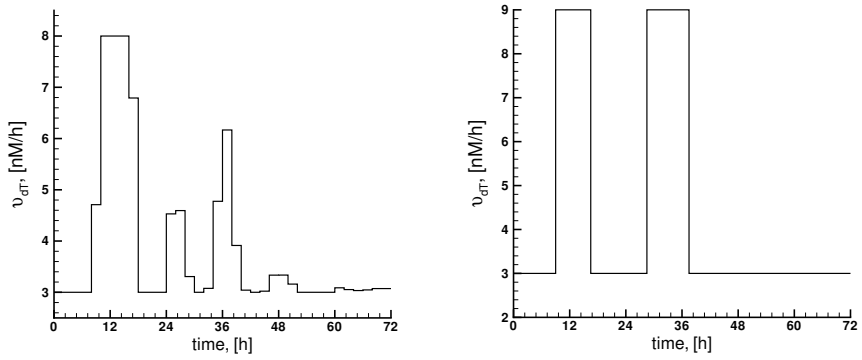

Figure 5: Relaxed (*left*) and bang-bang (*right*) optimal control functions for the phase tracking of circadian rhythms by light. The control input is the light-sensitive maximum rate of protein degradation $\nu_{\mathrm{dT}}(t)$.

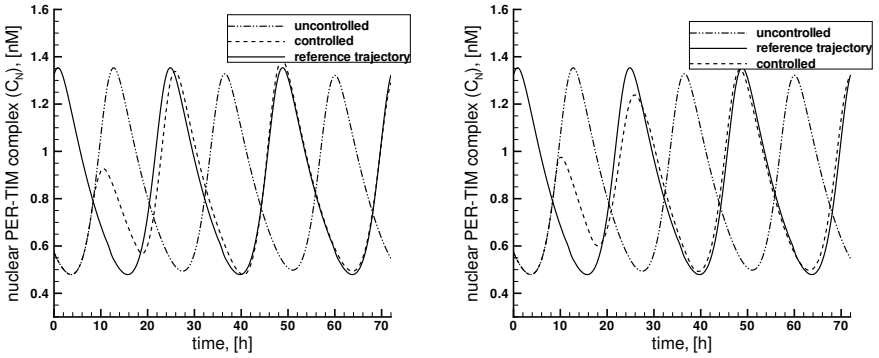

Figure 6: Phase tracking by light stimuli corresponding to the optimal control functions in figure 5, *left:* relaxed control scenario, *right:* bang-bang control scenario. The plot shows the PER-TIM protein concentration. Solid lines symbolize reference trajectories while dashed lines symbolize the controlled and uncontrolled PER-TIM complex.

mutant *Drosophila*, called per^s, with altered amplitude and endogenous oscillation period of 29 hours and 19 hours respectively. Such mutants with long period of about 29 hours and short period of about 19 hours are well known in case of *Drosophila* [34]. For optimal control, we consider the problem of shifting a mutant *Drosophila* PER cycle towards a wild-type *Drosophila* PER cycle, setting its period precisely back to 24 hours. Since we have changed for modeling

the mutant *Drosophila* PER cycles the light sensitive parameter ν_{dT}, we assume that the alteration of circadian rhythms have to be influenced by an indirect way of pharmalogical access to change the period and geometric characteristics of the altered limit cycle characterzing the nominal oscillations. We use the translation frequency k_{sP} and k_{sT} of PER and TIM proteins as control parameters, which may be influenced by suitable drugs. Time varying drug injecting pumps with constant inputs could be used for the purpose of chronomodulated drug administration. The control objective is to restore the nominal 24 hours period of circadian oscillations and the characteristics of the oscillations (shape and amplitude) close to the nominal values.
Starting from the mutant *Drosophila* per^l, we focus on changing the translation frequency k_s of mRNAs into the nonphosphorylated form of proteins. Here we assume the translation frequency of PER k_{sP} and TIM protein k_{sT} are the same and equal to k_s. In our case, k_s is assumed to switch between a minimal ($k_{\min}$) and a maximal ($k_{\max}$) value . This can be formulated as $k(t) = k_{\min} + w(t)(k_{\max} - k_{\min})$, where $w(t)$ is a binary-valued control function. In figure 7 (*left*), the resulting control input $k_s(t)$ as a solution of problem (1) using a piecewise constant control parametrization with relaxation of the integer constraints is shown. With this control function, the controller is able to restore a period of 24 hours, phase and amplitude of nominal oscillations very well for the long period mutant *per*^{l} by suitable variation of the translation frequency (data not shown). In figure 7 (*right*), the mixed integer solution of the optimal control problem (1) is shown, which has been computed starting from the relaxed control function. The obtained controlled trajectories are plotted in figure 8 for the mixed-integer control input. In the (*left*) plot, the controlled and uncontrolled per^l mRNA concentrations and the non mutant wild type state trajectory $\mathrm{x}_r(\mathrm{t})$ are shown. From this figure, it can be seen that it is possible to restore both, the period and amplitude of the mutant oscillations, close to the desired trajectory $\mathrm{x}_r(\mathrm{t})$. For visualisation, we plotted nuclear per mRNA *vs* PER-TIM complex concentration on right side of figure 8.

Similar results can be achieved for mutant type *Drosophila* per^s with altered amplitude and endogeneous oscillation period of 19 hours. Here, only the mixed integer control inputs $k_s(\mathrm{t})$ for restoration of per^s mutant rhythm are shown in figure 9 (*left*) and the corresponding controlled per mRNA trajectory (figure 9 (*right*)). The pulse control is also obtained starting from a former computed relaxed control function (data not shown). It is possible to restore the period of oscillations to 24 hours but the amplitude of oscillations are not restored well. Parameters with higher sensitivity, like transcription rates might allow more efficient ways of restoration in this case.

6 Conclusion

Applications of control theory to complex biological systems and model-based specific manipulation of system dynamics are promising visions in biomedical applications for systematic design of chronomodulated therapeutics. Since it is

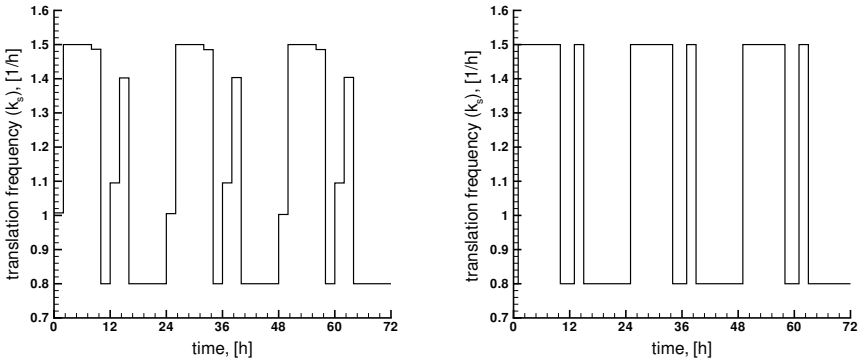

Figure 7: Optimal control for the relaxed problem (*left*) and the bang-bang problem (*right*) of circadian rhythm restoration of mutant per^l in *Drosophila* by varying the translation frequency k_s as control input.

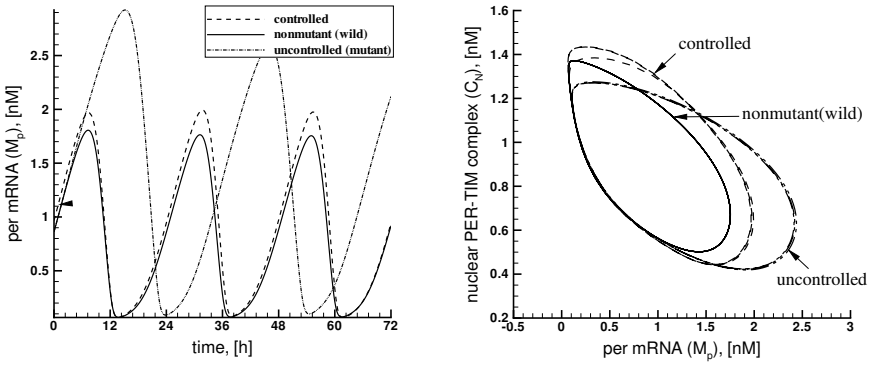

Figure 8: Rhythm restoration of mutant *Drosophila* per^l by the optimal bang-bang control functions from figure 7 (*right*). The (*left*) plot shows the per mRNA protein concentration and the (*right*) plot shows the corresponding limit cycles for the per^l mutant restoration in a phase space projection.

often not possible to control system dynamics in a continuous manner, systematic computations of discrete control functions are necessary. As an example to demonstrate the value of model based numerical optimal control in this context, a mixed-integer programming approach is applied to a *Drosophila* model to manipulate the system dynamics in a systematic way. Analysis of the circadian clock demonstrates that control inputs, such as light, that directly influences the parameter ν_{dT}, can be used to manipulate the system dynamics. Translation

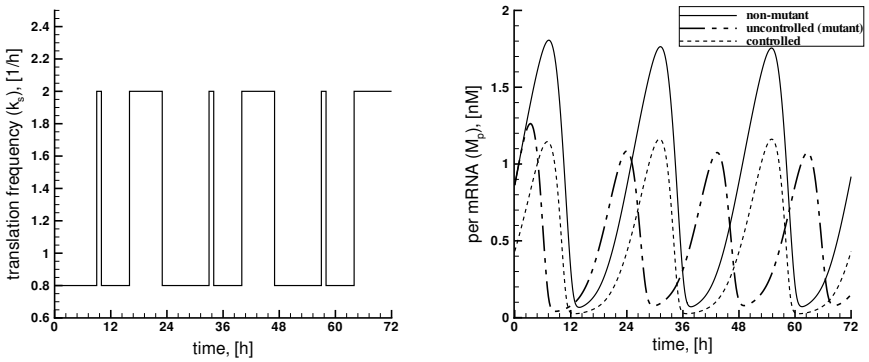

Figure 9: Bang-bang control solution (*left*) for circadian rhythm restoration of mutant per^s in *Drosophila* by varying the translation frequency k_s. The plot (*right*) shows the corresponding per mRNA protein concentration.

frequencies k_s may also be used as control functions, if they can be influenced in order to restore altered circadian rhythms. This may be done by suitable drugs, which mostly can only be applied in a discrete fashion. Although the study makes use of *Drosophila* circadian system, the phase tracking and restoration control algorithm described in this paper is generic and can be applied to any biological oscillator.

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A Drosophila model-equations and rate-constants

$$
\begin{aligned}
\frac{dM_{\mathrm{p}}}{dt} &= v_{\mathrm{sP}}\frac{{K_{\mathrm{IP}}}^{\mathrm{n}}}{{K_{\mathrm{IP}}}^{\mathrm{n}}+{C_{\mathrm{N}}}^{\mathrm{n}}} - v_{\mathrm{mP}}\frac{M_{\mathrm{P}}}{K_{\mathrm{mP}}+M_{\mathrm{P}}} - k_{\mathrm{d}}M_{\mathrm{P}} \\
\frac{dP_0}{dt} &= k_{\mathrm{sP}}M_P - V_{1\mathrm{P}}\frac{P_0}{K_{1\mathrm{P}}+P_0} + V_{2\mathrm{P}}\frac{P_1}{K_{2\mathrm{P}}+P_1} - k_{\mathrm{d}}P_0 \\
\frac{dP_1}{dt} &= V_{1\mathrm{P}}\frac{P_0}{K_{1\mathrm{P}}+P_0} - V_{2\mathrm{P}}\frac{P_1}{K_{2\mathrm{P}}+P_1} - V_{3\mathrm{P}}\frac{P_1}{K_{3\mathrm{P}}+P_1} + V_{4\mathrm{P}}\frac{P_2}{K_{4\mathrm{P}}+P_2} - k_{\mathrm{d}}P_1 \\
\frac{dP_2}{dt} &= V_{3\mathrm{P}}\frac{P_1}{K_{3\mathrm{P}}+P_1} - V_{4\mathrm{P}}\frac{P_2}{K_{4\mathrm{P}}+P_2} - k_3P_2T_2 + k_4C - v_{\mathrm{dP}}\frac{P_2}{K_{\mathrm{dP}}+P_2} - k_{\mathrm{d}}P_2
\end{aligned}
$$

$$
\begin{aligned}
\frac{dM_{\mathrm{T}}}{dt} &= v_{\mathrm{sT}} \frac{{K_{\mathrm{IT}}}^{\mathrm{n}}}{{K_{\mathrm{IT}}}^{\mathrm{n}} + {C_{\mathrm{N}}}^{\mathrm{n}}} - v_{\mathrm{mT}} \frac{M_{\mathrm{T}}}{K_{\mathrm{mT}} + M_{\mathrm{T}}} - k_{\mathrm{d}} M_{\mathrm{T}} \\
\frac{dT_0}{dt} &= k_{\mathrm{sT}} M_{\mathrm{T}} - V_{1\mathrm{T}} \frac{T_0}{K_{1\mathrm{T}} + T_0} + V_{2\mathrm{T}} \frac{T_1}{K_{2\mathrm{T}} + T_1} - k_{\mathrm{d}} T_0 \\
\frac{dT_1}{dt} &= V_{1\mathrm{T}} \frac{T_0}{K_{1\mathrm{T}} + T_0} - V_{2\mathrm{T}} \frac{T_1}{K_{2\mathrm{T}} + T_1} - V_{3\mathrm{T}} \frac{T_1}{K_{3\mathrm{T}} + T_1} + V_{4\mathrm{T}} \frac{T_2}{K_{4\mathrm{T}} + T_2} - k_{\mathrm{d}} T_1 \\
\frac{dT_2}{dt} &= V_{3\mathrm{T}} \frac{T_1}{K_{3\mathrm{T}} + T_1} - V_{4\mathrm{T}} \frac{T_2}{K_{4\mathrm{T}} + T_2} - k_3 P_2 T_2 + k_4 C - v_{\mathrm{dT}} \frac{T_2}{K_{\mathrm{dT}} + T_2} - k_{\mathrm{d}} T_2 \\
\frac{dC}{dt} &= k_3 P_2 T_2 - k_4 C - K_1 C + k_2 C_{\mathrm{N}} - k_{\mathrm{dC}} C \\
\frac{dC_N}{dt} &= k_1 C - k_2 C_{\mathrm{N}} - k_{\mathrm{dN}} C_{\mathrm{N}}
\end{aligned}
$$

The total (nonconserved) quantities of PER and TIM proteins, $\mathrm{P_t}$ and $\mathrm{T_t}$ are given by

$$
\begin{aligned}
P_{\mathrm{t}} &= P_0 + P_1 + P_2 + C + C_{\mathrm{N}} \\
T_{\mathrm{t}} &= T_0 + T_1 + T_2 + C + C_{\mathrm{N}}
\end{aligned}
$$

Kinetic parameter	Parameter value	Kinetic parameter	Parameter value
$\mathrm{v_{sP}}$	1 $\mathrm{nMh^{-1}}$	k_d	0.01$\mathrm{h^{-1}}$
$\mathrm{v_{sT}}$	1 $\mathrm{nMh^{-1}}$	$\mathrm{k_{dC}}$	0.01$\mathrm{h^{-1}}$
$\mathrm{v_{mP}}$	0.7 $\mathrm{nMh^{-1}}$	$\mathrm{k_{dN}}$	0.01$\mathrm{h^{-1}}$
$\mathrm{v_{mT}}$	0.7 $\mathrm{nMh^{-1}}$	$\mathrm{V_{1P}}$	8 $\mathrm{nMh^{-1}}$
$\mathrm{K_{mP}}$	0.2 nM	$\mathrm{V_{1T}}$	8 $\mathrm{nMh^{-1}}$
$\mathrm{K_{mT}}$	0.2 nM	$\mathrm{V_{2P}}$	1 $\mathrm{nMh^{-1}}$
$\mathrm{k_{sP}}$	0.9 $\mathrm{h^{-1}}$	$\mathrm{V_{2T}}$	1 $\mathrm{nMh^{-1}}$
$\mathrm{k_{sT}}$	0.9 $\mathrm{h^{-1}}$	$\mathrm{V_{3P}}$	8 $\mathrm{nMh^{-1}}$
$\mathrm{v_{dP}}$	2.4 $\mathrm{nMh^{-1}}$	$\mathrm{V_{3T}}$	8 $\mathrm{nMh^{-1}}$
$\mathrm{v_{dT}}$	2.4 $\mathrm{nMh^{-1}}$	$\mathrm{V_{4P}}$	1 $\mathrm{nMh^{-1}}$
$\mathrm{k_1}$	0.6 $\mathrm{h^{-1}}$	$\mathrm{V_{4T}}$	1 $\mathrm{nMh^{-1}}$
$\mathrm{k_2}$	0.2 $\mathrm{h^{-1}}$	$\mathrm{K_{4T}}$	2.0 nM
$\mathrm{k_3}$	1.2 $\mathrm{nM^{-1}h^{-1}}$	$\mathrm{K_{4P}}$	2.0 nM
$\mathrm{k_4}$	0.6$\mathrm{h^{-1}}$	$\mathrm{K_{3T}}$	2.0 nM
$\mathrm{K_{IP}}$	1.0 nM	$\mathrm{K_{3P}}$	2.0 nM
$\mathrm{K_{IT}}$	1.0 nM	$\mathrm{K_{2T}}$	2.0 nM
$\mathrm{K_{dP}}$	0.2 nM	$\mathrm{K_{2P}}$	2.0 nM
$\mathrm{K_{dT}}$	0.2 nM	$\mathrm{K_{1T}}$	2.0 nM
n	4	$\mathrm{K_{1P}}$	2.0 nM

Table 1: Rate constants for the *Drosophila* model.

References

[1] Goldbeter, A.: 'Computational approaches to cellular rhythms', *Nature*, 2002, **420**, pp. 238-245

[2] Young, M.W.: 'The molecular control of circadian behavioral rhythms and their entrainment in Drosophila', *Annu. Rev. Biochem.*, 1998, **67**, pp. 135-152

[3] Reppert, S.M., and Weaver,D.R.: 'Coordination of circadian timing in mammals', *Nature*, 2002, **418**, pp. 935-941.

[4] Rajaratnam, S.M., Arendt, J.: 'Health in a 24-h society', *Lancet.*, 2001, **358**, pp. 999-1005

[5] Dijk, D.J., Lockley, S.W.: 'Integration of human sleep-wake regulation and circadian rhythmicity', *J. Appl. Physiol.*, 2002, **92**, pp. 852-62.

[6] Francis, L.: 'Chronotherapeutics: the relevance of timing in cancer therapy', *Cancer Causes Control*, 2006, **17**, pp. 611-621.

[7] Francis, L., Ueli, S.: 'Circadian rhythms: Mechanisms and therapeutic implications', *Annu. Rev. Pharmacol.*, 2007, **47**, pp. 593-628.

[8] Ida, I., Elisabeth, F., Jens, R., Stephane, B., Athos, G.B., Stefano, I., Laurent, M., and Francis, L.: 'Improved tumor control through circadian clock induction by seliciclib, a cyclin-dependent kinase inhibitor', *Cancer Res.*, **66**, 2006, pp. 10720-10728.

[9] Goldbeter, A., Claude, D.: 'Time-patterned drug administration:Insights from a modeling approach', *Chronobiol. Int.*, 2002, **19**, pp. 157-175.

[10] Francis, L.: 'Cancer chronotherapy' *Lancet Oncol.*, 2001, **2**, pp. 307-315.

[11] Mormont, M.C., Waterhouse, J., Bleuzen, p., Giachetti, S., Jami, A., Bogdan, A., Lellouch, J., Misset J. L., Touitou, Y., and Francis, L.: 'Marked 24 h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status', *Clin. Cancer Res.*, 2000, **6**, pp. 3038-3045.

[12] Loning, F., and Cheng, C. L.: 'The circadian clock: Pacemaker and tumour suppressor', *Nature*, 2003, **3**, pp. 350-361.

[13] Claude D., and Clairambault, J.: 'Period shift induction by intermittent stimulation in a drosophila model of per protein oscillations', *Chronobiol.Int.*, 2000, **17**, pp. 1-14.

[14] Laroche, b., and Claude, D: 'Flatness-based control of PER protein oscillations in a Drosophila model', *IEEE transactions on automatic control*, 2004, **49**, pp. 175-183.

[15] Boulos, Z., Macchi, M. M., Stürchler, M.P., Stewart, K.T., Brainard, G.C., Suhner, A., Wallace, G., and Steffen, R.: 'Light visor treatment for jet lag after westward travel across six time zones', *Aviat. Space. Environ. Med.*, 2002, **73**, pp. 953-963.

[16] Jewett, M.E., Kronauer, R.E., Czeisler, C.A.: 'Light-induced suppression of endogenous circadian amplitude in humans', *Nature*, 1991, **350**, pp. 59-62.

[17] Bauer, I., Finocchi, F., Duschl, W.J., Gail, H.P., Schlöder, J.P., 'Simulation of chemical reactions and dust destruction in protoplanetary accretion discs', *Astronomy and Astrophysics*, 1997, **317**, pp. 273-289.

[18] Slaby, O., Sager, S., Shaik, O., Kummer, U., Lebiedz, D.: 'Optimal control of self-organized dynamics in cellular signal transduction', *Math. and comp. modeling of dynamical systems*, 2007, accepted.

[19] Lebiedz, D., and Brandt-Pollmann, U.: 'Manipulation of Self-Aggregation Patterns and Waves in a Reaction-Diffusion System by Optimal Boundary Control Strategies', *Physical Review Letters*, 2003, **91**, pp. 208301.

[20] Lebiedz, D., Sager, S., Bock, H. G., and Lebiedz, P.: 'Annihilation of limit cycle oscillations by identification of critical perturbing stimuli via mixed-integer optimal control', *Physical Review Letters*, 2005, **95**, pp. 108303.

[21] Leineweber, D. B., Bauer, I., Bock, H. G. and Schlöder, J. P.: 'An efficient multiple shooting based reduced SQP strategy for large-scale dynamic process optimization. Part I: Theoretical aspects', *Computers and Chemical Engineering*, 2003, **27**, pp. 157–166.

[22] Leloup, J. C., and Goldbeter, A.: 'A model for circadian rhythms in Drosophila incorporating the formation of a complex between the PER and TIM proteins', *Journal of Biological Rhythms*, 1998, **13**, pp. 70-87.

[23] Bock, H. G., and Plitt, K. J.: 'A multiple shooting algorithm for direct solution of optimal control problems', In: Proceedings 9th IFAC World Congress Budapest, 1984, Pergamon, pp. 243-247.

[24] Sager, S.: 'Numerical methods for mixed–integer optimal control problems', (Der andere Verlag, Tönning, Lübeck, Marburg, 2005)

[25] Winfree, A. T.: 'The geometry of biological time', (Springer, New York, 2001)

[26] Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A., and Young, Y. M.: 'Light-induced degradation of TIMELESS and entrainment of the Drosophila circadian clock', *Science.*, 1996, **271**, pp. 1736-1740.

[27] Zeng, H., Qian, Z., Myers, M. P., and Rosbash, M.: 'A lightentrainment mechanism for the Drosophila circadian clock', *Nature.*, 1996, **380**, pp. 129-135.

[28] Alper, H., Fischer, C., Nevoigt, E., and Stephanopoulos, G.: 'Tuning genetic control through promoter engineering', *Proc. Natl. Acad. Sci. USA.*, 2005, **102**, pp. 12678-12683.

[29] de Smit, M. H., and van Duin J.: 'Secondary structure of the ribosome binding site determines translational efficiency: a quantitative analysis', *Proc. Natl. Acad. Sci. USA.*, 1990, **87**, pp. 7668-7672.

[30] Smolke, C. D., T. A. Carrier, and J. D. Keasling: 'Coordinated, differential expression of two genes through directed mRNA cleavage and stabilization by secondary structures', *Appl. Environ. Microbiol.*, 2000, **66**, pp. 5399-5405.

[31] Leloup, J.C.; Goldbeter, A.: 'Modeling the Molecular Regulatory Mechanism of Circadian Rhythms in Drosophila', *BioEssays*, 2000, **22**, pp. 84-93.

[32] Ruoff, P., Vinsjevik, M., Monnerjahn, C., Rensing, L.: 'The Goodwin Model: Simulating the Effect of Light Pulses on the Circadian Sporulation Rhythm of Neurospora crassa', *J. Theor. Biol.*, 2001, **209**, pp. 29-42.

[33] Doedel, E.J.: 'A program for the automatic bifurcation analysis of autonomous systems', *Congr. Num.*, 1981, **30**, pp. 265-284.

[34] Ronald, J. K., and Seymonr, B.: 'Clock mutants of Drosophila Melanogaster', *Proc. Natl. Acad. Sci. USA.*, 1971, **68**, pp. 2112-2116.