Equilibrium Bifurcation Analysis of Nonlinear Biochemical Systems using Control-based Continuation and Model Predictive Control

Irene de Cesare*, Lucia Marucci*,+ and Ludovic Renson †,+
*Engineering Mathematics Department, University of Bristol, UK.
†Department of Mechanical Engineering, Imperial College London, UK.
+ Co-senior and co-corresponding authors; l.renson@imperial.ac.uk; lucia.marucci@bristol.ac.uk

Summary. Control-based continuation (CBC) is a testing method that maps the nonlinear dynamic features of a physical system directly from experiments. The application of CBC has been so far limited to electro-mechanical systems. In this paper, we numerically investigate the application of CBC to a synthetic gene network, the toggle switch. The network's behaviour is simulated using a nonlinear model (with and without noise), and model predictive control (MPC) is exploited to control its dynamics, through the definition of an appropriate control objective function. Our preliminary results show that MPC-based CBC can accurately capture the continuation curve obtained numerically on the full nonlinear model.

Introduction

Mathematical modelling is widely used in System and Synthetic Biology to understand nonlinear biochemical phenomena (e.g. gene expression temporal oscillations), and to design and validate engineered gene circuits. Although widely used, biochemical models can be challenging in both their derivation and associated parameter identification [1, 2]. Parameter and model uncertainty can significantly alter the reliability of model predictions of nonlinear dynamic behaviours.
The application of control-based continuation (CBC) to biological systems, not attempted to date, has the potential to overcome these difficulties, improving the understanding of naturally-occurring nonlinear biochemical dynamics (e.g. oscillations in signalling pathways) and enabling the rapid prototyping of engineered gene regulatory network dynamics. CBC is a non-parametric method that combines feedback control with principles of numerical continuation to map out the dynamic features of a nonlinear physical system directly during experimental tests [3]. The fundamental principles of CBC are well established and the method has been applied to a wide range of non-living (i.e. electro-mechanical) systems [4, 5]. In this contribution, we investigate the use of CBC to detect and track the equilibria of the toggle switch, an engineered genetic circuit [6]. Model-predictive control (MPC), previously exploited to control biological systems [7], is employed to steer the system's dynamics towards steady-state and stabilise unstable responses of the underlying uncontrolled system.

Control-Based Continuation of a synthetic toggle switch's dynamics

We investigate the application of CBC on a toggle switch, one of the first and best characterised synthetic gene network implemented to date in living bacterial cells [6, 8]. The gene network consists of two repressors ($LacI$ and $TetR$) and two inducers (Atc and $IPTG$). Each repressor affects the opposite gene's production, which can be externally tuned through the addiction of chemical inducers. Levels of $LacI$ and $TetR$ can be observed thanks to fluorescent reporters (Figure 1A). The associated deterministic model is mathematically described by a set of four nonlinear differential equations taking into account mRNA transcription and translation of both repressors. For the stochastic scenario, a SDE model for the description of biochemical systems based on pseudo-reactions is considered [9].
We chose Model-Predictive Control (MPC) as control strategy to implement CBC; this method combines state estimation, model prediction and optimization algorithms to find the optimal actuation to apply to a system. At each time step i, the choice of the optimal sequence of N control inputs is based on the minimization of the cost function

$$J_i = \sum_{k=1}^{N} (N-k+1)e(i+k)^2, \tag{1}$$

where the error $e(i+k)$ is defined as the difference between the control target and the prediction of the system's response over N samples. The system response to a particular sequence of control inputs is predicted by integrating in time a linear model of the system. This linear model was identified prior to closed-loop simulations using input-output *in-silico* data and time-domain subspace algorithms. At each time step i, the initial conditions required to initialise simulations are obtained using a Kalman filter that was also derived during the linear identification process. When an optimal sequence of inputs is found, only the actuation for the next time step is applied to the system. This whole optimization process is repeated at each actuation time step i.
Cost function (1) usually includes an additional term to balance tracking performance and control effort. This term is not considered here but replaced by optimization constraints limiting the control action u at time step i to be no more than 30% different from the control action at time step $i-1$.
The inducer $IPTG$ is considered to control the toggle switch's dynamics. IPTG regulates the expression of a fluorescent reporter gene, which is a proxy for the $TetR$ concentration (see Fig. 1A). The controller based on the optimization of Eq. (1) with constraints allows to reach any meaningful value of the $TetR$ concentration. At steady state, the value of the control input ($IPTG$) can be interpreted as the bifurcation parameter [10]. Recording $IPTG$ for a range of target

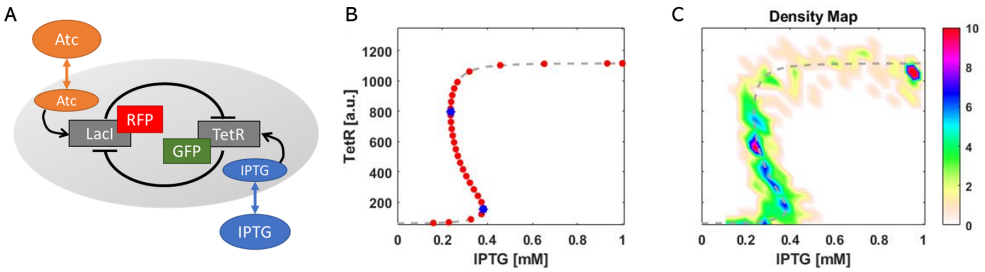

Figure 1: MPC-based CBC of a synthetic toggle switch. A) Schematic representation of the toggle switch, adapted from [6], where RFP and GFP represent the fluorescent proteins binding with $LacI$ and $TetR$. B) CBC results and numerical continuation bifurcation diagram (COCO software) are shown as red points ● and a dotted line (——), respectively; blue points ● design the bifurcation points. $IPTG$ is the inducer, used as bifurcation parameter. C) CBC results on the stochastic model are represented as a density plot: 10 repetitions of the same experiment are run and 300 points are collected.

concentrations, the equilibrium curve of the uncontrolled toggle switch can be traced out. Fig. 1B shows the excellent agreement obtained between bifurcation diagram computed using standard numerical continuation algorithms (dashed line) and CBC results (red dots). Saddle-node bifurcations are highlighted in blue in Fig. 1B. For the stochastic case, multiple simulations are considered and 300 points are collected (Fig. 1C).

Conclusions

This paper investigates the use of CBC for tracking the equilibrium of synthetic gene regulatory networks. Our preliminary *in silico* results demonstrate the feasibility of the approach and the ability of CBC to capture both the stable and unstable behaviours of the original, uncontrolled system. In particular, in the absence of noise, the equilibrium curve measured using CBC perfectly agrees with the one computed using standard numerical continuation. In the stochastic scenario, CBC is still able to uncover the bistable nature of the system as data points qualitative follow the equilibrium curve of the underlying deterministic model. Unstable steady states collected using CBC also prove to be beneficial when estimating model parameters that reproduce bistability. The introduction of CBC to characterise cellular dynamics could benefit both the System and Synthetic Biology communities, providing a valuable tool to explore complex nonlinear dynamics and to gather important information that can eventually enable a more precise prototyping of those dynamics into novel synthetic gene circuits. The application of CBC to biochemical experiments could be done using microfluidics/microscopy platforms for real-time monitoring and control of gene expression in living cells via dynamic modulation of inducer molecules [11].

Acknowledgements

IdC is supported by an EPSRC DTP Scholarship. LM is supported by the Medical Research Council grant MR/N021444/1, by the Engineering and Physical Sciences Research Council grants EP/R041695/1 and EP/S01876X/1, and by the European Union's Horizon 2020 under Grant Agreement No. 766840. LR is funded by a Research Fellowship from the Royal Academy of Engineering (RF1516/15/11).

References

[1] Marucci, L. (2017). Nanog dynamics in mouse embryonic stem cells: results from systems biology approaches. Stem cells international.

[2] Banga J. R.(2008). Optimization in computational systems biology. BMC systems biology.

[3] Sieber, J., Krauskopf, B. (2008). Control based bifurcation analysis for experiments. Nonlinear Dynamics, 51(3), 365-377.

[4] Renson L., Barton D.A., Neild S.A. (2017). Experimental tracking of limit-point bifurcations and backbone curves using control-based continuation. International Journal of Bifurcation and Chaos.

[5] Renson L., Shaw A. D., Barton, D. A. W., Neild, S. A. (2019). Application of control-based continuation to a nonlinear structure with harmonically coupled modes. Mechanical Systems and Signal Processing.

[6] Lugagne J.B., Carrillo S.S., Kirch, M., Köhler, A., Batt, G., & Hersen, P. (2017). Balancing a genetic toggle switch by real-time feedback control and periodic forcing. Nature communications, 8(1), 1671.

[7] Postiglione L.; Napolitano S.; Pedone E.; Rocca D. L.; Aulicino, Francesco; Santorelli, Marco; Tumaini, Barbara; Marucci, Lucia; di Bernardo, Diego. (2018). Regulation of gene expression and signaling pathway activity in mammalian cells by automated microfluidics feedback control. ACS synthetic biology.

[8] Gardner T.S., Cantor C.R., Collins J.J. (2000). Construction of a genetic toggle switch in Escherichia coli. Nature.

[9] Lakatos, E. (2017). Stochastic analysis and control methods for molecular cell biology.

[10] Barton D. A., Sieber J. (2013). Systematic experimental exploration of bifurcations with noninvasive control. Physical Review E, 87(5), 052916.

[11] Menolascina F., Fiore G., Orabona E., De Stefano L., Ferry M., Hasty J., ... & di Bernardo, D. (2014). In-vivo real-time control of protein expression from endogenous and synthetic gene networks. PLoS computational biology, 10(5), e1003625.