Stochastic modeling of a tunable synthetic mammalian oscillator

Tatiana T. Marquez-Lago[1,2], Marcel Tigges[1], Martin Fussenegger[1] and Jörg Stelling[1,2]

Short Abstract — **One of the central topics in molecular biology consists of understanding biological rhythms and discerning whether it is possible to influence the underlying genetic clockwork to tune the expression of key genes. Answering this question may prove to be central in the design of future gene therapies, particularly those requiring a periodic input. For such purpose, we designed a synthetic regulatory network that provides tunable oscillating gene expression in mammalian cells [1]. In this talk, we will focus on the stochastic modeling that provided us with sufficient conditions for tuning gene expression.**

***Keywords* — Stochastic simulations, tunable gene expression, oscillators.**

I. Background

CIRCADIAN clocks have intriguing dynamics and play important roles in controlling critical repair, metabolic and signaling pathways. Since their underlying molecular mechanisms and expression dynamics are still not fully understood, we constructed and modeled a synthetic mammalian clock, providing a new insight into the dynamics of natural periodic processes [1].

The synthetic mammalian oscillator is based on an auto-regulated sense–antisense transcription control circuit encoding a positive and a time-delayed negative feedback loop in a compact manner, enabling autonomous, self-sustained and tunable oscillatory gene expression. Our systems design relied on both experimental analyses and mathematical modeling, upon which we monitored oscillating concentrations of green fluorescent protein with tunable frequency and amplitude by time-lapse microscopy in real time in individual Chinese hamster ovary cells. The experimental findings were accurately predicted by our mathematical models, providing sufficient conditions for tuning mammalian gene expression.

Acknowledgements: This work was supported by the Swiss National Science Foundation and the EC Framework 6 (COBIOS).

[1] Department of Biosystems Science and Engineering, Swiss Federal Institute of Technology (ETH) Zurich, Mattenstrasse 26, CH-4058 Basel, Switzerland. E-mail: tatiana.marquez@bsse.ethz.ch

[2] Swiss Institute of Bioinformatics, ETH Zurich, CH-8092 Zurich, Switzerland.

II. Mathematical and Computational Methods

As a first step, we constructed a deterministic model that described the time evolution of the concentration of two regulators (tTA and PIT) and the green fluorescent protein as well as the interactions between them, considering the processes of transcription, translation and degradation individually. Within the observed experimental constraints we conducted simulation studies with varying model parameters and/or inputs to establish critical design features, obtaining a structurally refined model that could describe all observed experimental data quantitatively. Since previous studies pointed to stochasticity as an accuracy bottleneck in synthetic networks [2-3], we finally established a detailed stochastic model that allowed us to estimate the impact of molecular noise on the synthetic mammalian oscillator.

Our analysis included the construction of the mathematical model from scratch and a parameter estimation through an evolutionary strategy for data not found in the literature. We also devised computational methods for analyzing single-cell data, such as alignment of single-cell fluorescence trajectories and unbiased estimation of oscillation periods and amplitudes from noisy time series, i.e. our monitored oscillating concentrations of green fluorescent protein obtained *in vitro* and *in silico*.

III. Conclusion

Our simulations accurately predicted that, even when the ratios of oscillator components were fixed, absolute plasmid concentrations could be used to alter the period and amplitude of the oscillations. Our stochastic simulations showed that noise contributes substantially to the observed cell-to-cell variability in the period and, for low plasmid dosages, the model predicts that not all cells will oscillate. Moreover, for high plasmid dosages, we found low-amplitude noise-induced oscillations that are similar to those predicted for simplified oscillators [3]. This underlines the importance of stochastic effects on the quantitative behavior of systems with complicated dynamics.

References

[1] Tigges M., Marquez-Lago T.T., Stelling J. and Fussenegger M. (2009) A tunable synthetic mammalian oscillator. *Nature* **457**, 309-312.

[2] Rosenfeld N. et al. (2005) Gene regulation at the single-cell level. *Science* **307**, 1962-1965.

[3] Vilar J.M. et al. (2002) Mechanisms of noise-resistance in genetic oscillators. *PNAS* **99**, 5988-5992.