# BIOMOLECULAR FEEDFORWARD LOOPS: PULSE DYNAMICS, DESIGN CONSTRAINTS, AND ROBUSTNESS

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Understanding and designing of systems-level behaviours such as pulse dynamics is an important task in systems and synthetic biology. Pulse dynamics appears in multiple instances in biology, from the molecular scale of gene regulation to the population scale of ecology and epidemiology. In synthetic biology, these dynamical behaviours are implemented by circuits of interacting biomolecules. Significant advances have been made to understand the underlying dynamical principles, especially in natural biological systems and to implement these key properties such as robustness towards changes in the environmental conditions, in synthetically designed circuits. Further, understanding design constraints have been helpful in predicting achievable performance in the synthetic circuits. However, the design issues to achieve the desired behaviour in a biomolecular circuit with pulse dynamics are relatively unclear. To address these, we used a combination of mathematical models and experimental measurements of a pulse-generating biomolecular circuit. We used the incoherent feedforward loop as an example of a pulse-generating biomolecular circuit. In this context, we investigated three design aspects — the underlying mechanism of the pulse, the constraints in the achievable performance of the pulse, and the robustness of the pulse towards the environmental variable of temperature. We found that non-normality, which constrains the output solution to evolve as a difference of exponentials, could facilitate pulsing in an incoherent feedforward loop. Based on this, we developed frameworks to quantitate pulse behaviours, to screen pulse behaviours in arbitrary networks, and to design pulses adhering to given specifcations. We examined the constraints, specifically the co-variation of amplitude and timescale, in a pulse experimentally, and found that larger amplitude pulses had slower rise time. We characterized these trends in computational models and discussed the experimental results in the context of the mathematical models. We assessed the extent of robustness in a pulse to the perturbation of temperature experimentally and found that the change in the amplitude and the timescale of the pulse depended upon the temperature and might relate to the change in the growth rate of the host cell. We noted that the matching temperature dependencies of the parameters in the mathematical model could cancel each other's effect and exhibit overall temperature robustness. Further, we developed a control-theoretic framework using contraction theory and finite-time Lyapunov exponents to enhance robustness to temperature in a biomolecular circuit and validated this framework using computational models. Overall, this investigation should facilitate the analysis and the design of systems-level dynamics in biology.