

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

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# Biomolecular Feedforward Loops: Pulse Dynamics, Design Constraints, and Robustness

*by*

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# CERTIFICATE

This is to certify that the thesis entitled **Biomolecular Feedforward Loops: Pulse Dynamics, Design Constraints, and Robustness** submitted by **Abhilash Patel** to the Indian Institute of Technology Delhi, for the award of the Degree of **Doctor of Philosophy**, is a record of the bonafide research work carried out by him under my supervision and guidance. The thesis has reached the standards fulfilling the requirements of the regulations relating to the degree.

The results contained in this thesis have not been submitted either in part or in full to any other University or Institute for the award of any degree or diploma to the best of my knowledge.

Prof. Shaunak Sen

Department of Electrical Engineering,

Indian Institute of Technology Delhi.

(Supervisor)

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# ABSTRACT

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 specifications. 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.

## सार

सिस्टम-स्तरीय व्यवहार जैसे पल्स डायनामिक्स को समझना और डिजाइन करना सिस्टम्स और कृत्रिम जीव विज्ञान में एक महत्वपूर्ण कार्य है। पल्स डायनामिक्स जीव विज्ञान में कई उदाहरणों में प्रकट होता है, जीन विनियमन के आणविक पैमाने से लेकर पारिस्थितिकी और महामारी विज्ञान के जानसंख्यिक पैमाने तक। कृत्रिम जीव विज्ञान में, इन गतिशील व्यवहारों को परस्पर बायोमोलेक्यूलर्स के सर्किट द्वारा कार्यान्वित किया जाता है। अंतर्निहित गतिशील सिद्धांतों को समझने के लिए, विशेष रूप से प्राकृतिक जैविक प्रणालियों में, और प्रमुख गुणों, जैसे कि पर्यावरणीय परिस्थितियों में परिवर्तन के प्रति मजबूती, को कृत्रिम रूप से डिजाइन किए गए सर्किट में लागू करने के लिए महत्वपूर्ण प्रगति की गई है। इसके अलावा, डिजाइन की बाधाओं को समझना कृत्रिम सर्किट व्यवहार की भविष्यवाणी करने में मददगार रहा है। हालांकि, पल्स डायनामिक्स के साथ बायोमोलेक्यूलर सर्किट में वांछित व्यवहार को प्राप्त करने के लिए डिजाइन के मुद्दे अपेक्षाकृत अस्पष्ट हैं। इन्हें संबोधित करने के लिए, हमने गणितीय मॉडल के संयोजन और एक पल्स-जनरेटिंग बायोमोलेक्यूलर सर्किट के प्रयोगात्मक माप का उपयोग किया। हमने पल्स-जनरेटिंग बायोमोलेक्यूलर सर्किट के उदाहरण के रूप में असंगत फीडफॉरवर्ड लूप का उपयोग किया। इस संदर्भ में, हमने तीन डिजाइन पहलुओं की जांच की - पल्स की अंतर्निहित तंत्र, पल्स के प्राप्त करने योग्य व्यवहार में बाधाएं, और तापमान के पर्यावरणीय चर के प्रति पल्स की मजबूती। हमने पाया कि नॉन-नोर्मलिटी, जो आउटपुट समाधान को एक्सपोनेंटीकल्स के अंतर के रूप में विकसित करने के लिए बाधित करती है, एक असंगत फीडफॉरवर्ड लूप में पल्स की सुविधा प्रदान कर सकती है। इसके आधार पर, हमने पल्स व्यवहार की मात्रा निर्धारित करने के लिए, मनमाने नेटवर्क में पल्स व्यवहार को स्क्रीन करने के लिए, और दिए गए विनिर्देशों का पालन करने वाले दालों को डिजाइन करने के लिए रूपरेखा विकसित की। हमने प्रयोगात्मक रूप से एक पल्स में बाधाओं, विशेष रूप से आयाम और समय के सह-भिन्नता की जांच की, और पाया कि बड़े आयाम वाले पल्स में धीमी वृद्धि का समय था। हमने कम्प्यूटेशनल मॉडल में इन प्रवृत्तियों की विशेषता बताई और गणितीय मॉडल के संदर्भ में प्रयोगात्मक परिणामों पर चर्चा की। हमने प्रयोगात्मक रूप से तापमान की गड़बड़ी के लिए एक पल्स में मजबूती की सीमा का आकलन किया और पाया कि पल्स के आयाम और समय में परिवर्तन तापमान पर निर्भर करता है और मेजबान सेल की वृद्धि दर में परिवर्तन से संबंधित हो सकता है। हमने नोट किया कि गणितीय मॉडल में मापदंडों की मिलान तापमान निर्भरता एक दूसरे के प्रभाव को रद्द कर सकती है और समग्र तापमान मजबूती प्रदर्शित कर सकती है। इसके अलावा, हमने बायोमोलेक्यूलर सर्किट में तापमान की मजबूती को बढ़ाने के लिए संकुचन सिद्धांत और परिमित-समय ल्यापुनोव प्रतिपादकों का उपयोग करके एक नियंत्रण-सैद्धांतिक ढांचा विकसित किया और कम्प्यूटेशनल मॉडल का उपयोग करके इस ढांचे को मान्य किया। कुल मिलाकर, इस जांच से जीव विज्ञान में सिस्टम-स्तरीय गतिकी के विश्लेषण और डिजाइन की सुविधा मिलनी चाहिए।



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