

Gene expression systems that are inducible offer control over the expression of a target gene using a small molecule. One common approach involves engineering a transcription factor (TF) responsive to a small molecule, along with its corresponding promoter. However, this design often faces challenges in balancing minimal uninduced background expression (leakiness) with maximal induced expression. In this study, an alternative strategy is explored using quantitative synthetic biology to mitigate leakiness while maintaining high expression without modifying either the TF or the promoter. The focus of the research is the development of the CASwitch, a mammalian synthetic gene circuit designed through a combination of two well-known network motifs: the Coherent Feed-Forward Loop (CFFL) and the Mutual Inhibition (MI). This innovative approach involves combining the CRISPR-Cas endoribonuclease CasRx with the Tet-On3G inducible gene system to achieve optimal performance. The CASwitch is designed through mathematical modeling and validated experimentally. To showcase the potential of the CASwitch, it is applied to three distinct scenarios: enhancing a whole-cell biosensor, controlling the expression of a toxic gene, and inducing the production of Adeno-Associated Virus (AAV) vectors. The results demonstrate the versatility and efficacy of the CASwitch in diverse applications, emphasizing its value in synthetic biology and controlled gene expression systems.