# Designing Novel Synthetic Genetic Oscillators in Yeast Using Protein Sequestration

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***Short Abstract* — Genetic oscillators play important roles in natural systems (e.g. cell cycle, circadian rhythms). The synthetic genetic oscillators built so far have neglected protein sequestration, a core regulatory mechanism in natural oscillators. Here, we propose two small genetic oscillators that utilize protein sequestration, and we evaluate the possibility of their implementation in yeast. We show that the delay in DNA-transcription factor association/dissociation step is critical for sustainable oscillators in our systems, a step that is often overlooked in theoretical modeling. We also modeled the intrinsic noise in our systems using Gillespie simulation and characterized the entrainment properties of our oscillators.**

***Keywords* — Synthetic oscillator, protein sequestration, budding yeast**

## I. Introduction

SYNTHETIC genetic oscillators are powerful tools for predicting and understanding the dynamical properties of the natural genetic oscillatory networks. However, so far, synthetic oscillators have neglected protein sequestration, which is a core regulatory mechanism in many natural oscillators [1,2]. Our first synthetic design is based on the Mixed Feedback Loop (MFL) [3], which consists of an activator that activates its own inhibitor, which, in turn, sequesters the activator into an inactive complex (Fig.1). However, the use of activators in genetic circuits has been challenging due to difficulties in controlling the activation strength and/or toxicity (i.e. squelching). Therefore, we propose a novel variation of the MFL, the Double-negative Feedback Loop (DFL) that consists of a repressor, which acts on its own promoter and constitutively expressed inhibitor that forms inactive complex with the repressor (Fig.1). This design uses only repression and is free from experimental problems of activators.

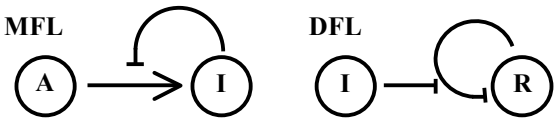


**Fig. 1**

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## II. Methods and Results

We analyzed "realistic" mathematical models of the MFL and DFL and performed numerical simulations to assess the plausibility of MFL and DFL oscillators in yeast and to understand the effects of the molecular noise on our systems.

### *A. Numerical Deterministic Simulations*

We restrict our parameters to specific physiological range for budding yeast and the proteins we intend to use (basic leucine zippers: bZIPs). By exhaustively searching through parameter space, we checked whether there are any restrictions for the oscillatory solutions. We found that the main restriction is on the DNA- repressor (activator) association/dissociation rates, since the delays in DNA binding/unbinding steps are key pacemakers of our systems. This poses a potential challenge since bZIPs tend to have fast DNA binding/unbinding rates. We show that this restriction can be alleviated by the addition of extra repressor (activator) binding sites, which decreases the effective DNA unbinding rate.

### *B. Numerical Stochastic Simulations*

We used the direct Gillespie algorithm to simulate the intrinsic noise of the systems caused by molecular fluctuations and discrete binding events of transcription factors to the DNA. Our simulations suggest that this intrinsic noise would cause our oscillators to lose synchrony almost immediately. However, the system was entrained very effectively by the simulated square wave driving signal.

## III. Conclusions and Future Work

We are currently trying to build those circuits using our theoretical work as a guide. Overall, numerical simulations strongly suggest the possibility of the implementation MFL and DFL oscillators in yeast using our proteins of choice.

## References

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