Synthetic NF-κB: A Building Approach to Study Complex Signaling Behaviors

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***Short Abstract* — The precise dynamic features in cell signaling play crucial roles in regulating various cellular functions. Due to the complexity and redundancy in natural cells, it remains challenging to completely understand how the complex temporal behaviors are programmed in parameters or structures of the signaling circuits. To overcome such problems, we took a synthetic approach to reconstitute the human nuclear factor κB (NF-κB) system in S. cerevisiae. This simple but highly tunable circuit allows us to systematically explore the design principles of oscillatory signaling dynamics.**

***Keywords* — Signaling Dynamics, NF-κB, Oscillation, Waveform, Frequency, Circuit, Negative Feedback, Synthetic Biology.**

Quantitative features of cellular signaling behaviors have drawn much attention due to their capabilities to carry extra information to regulate comprehensive downstream cellular events [1-2]. More recently, it has been found that different temporal behaviors or signaling dynamics of Msn2 in yeast and p53 in mammals resulted in dramatically different cell fates [3-5]. Remarkably, such quantitative signaling behaviors were often oscillatory or pulsatile. The particular properties of oscillatory signaling dynamics (e.g., amplitude, frequency) have shown great benefit to coordinate or differentially regulate gene expression in stress signaling and inflammatory response [6]. It remains incompletely understood how the properties of such oscillatory dynamics are controlled in natural systems and how they could be tailored synthetically.

The NF-κB in immune response is one of the well-known signaling systems to be highly dynamic, responding to various extracellular antigens or cytokines. Notably, the fluorescence tagged NF-κB protein was found to be activated in a damped oscillatory fashion in response to tumor necrosis factor α (TNF-α) [7-8]. The core design of the circuit underlying this oscillator is similar to that of many biological oscillators; a negative feedback loop with time delay, usually consisting of a transcription factor (NF-κB) and an inhibitory protein (IκB). However, in practice, because the native NF-κB system exists in the complicated context of mammalian cells, it is difficult to specifically experimentally probe and rewire the circuit components of such oscillatory signaling networks.

Here, we designed and built a robust and tunable synthetic oscillatory signaling circuit in *Saccharomyces cerevisiae*. By recapitulating a human NF-κB module in yeast, the design emphasized orthogonality and predictable tuning performances. Additionally, the circuit contains a synthetic promoter module that can program oscillatory dynamics at the transcriptional level and importantly also contains a synthetic phospho-degron module that allows for programming at the post-translational level. We initially operated the circuit with a single negative feedback loop and found that the peak shape and the period of the oscillatory waveforms could be tuned using a combination of three parameters, including the protein level of RelA, negative feedback strength, and the protein stability of IκBα. Guided by model prediction, we next found a unique circuit structure with two-layers of negative feedback loops enabled frequency-only tuning of oscillatory waveforms.

We conclude that the signaling dynamics in such synthetic NF-κB circuit could be quantitatively customized through carefully selected circuit parameters and circuit structures. We propose that the design principles established here enable function-guided design of signaling controllers to meet the requirement of the increasing sophisticated and precise regulation of diverse synthetic biology applications.

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